

**POLYPHENOLS FROM *PERICOPSIS ELATA*
AND SYNTHESIS OF SELECTED STILBENES**

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TABLE OF CONTENTS

Summary	i
Opsomming	iii

CHAPTER 1. INTRODUCTION 1

LITERATURE SURVEY

CHAPTER 2. PHENOLIC COMPOUNDS

2.1. Introduction	3
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CHAPTER 3. FLAVONOIDS

3.1. Introduction	6
3.2. Structure and classes of flavonoids	7
3.2.1. Introduction	7
3.3. Flavanones and dihydrochalcones	9
3.3.1. Introduction	9
3.3.2. Structures of flavanones and dihydrochalcones	9
3.3.3. Identification by spectral methods	11
3.3.3.1. Nuclear magnetic resonance spectroscopy	11
3.3.4. Natural distribution	12
3.3.5. Biological and pharmacological properties	13
3.4. Isoflavones and α -methyldeoxybenzoin	13
3.4.1. Characterization	15
3.4.1.1. Nuclear magnetic resonance spectroscopy	15
3.4.2. Natural distribution	15
3.4.3. Biological Properties	16

3.5.	Biosynthesis	17
3.5.1.	Flavanones, chalcones and isoflavonoids	17
3.6.	Chemistry of the flavonoids	20
3.6.1	Chemical structure	20
3.6.2.	Structural features and antioxidant activity	20
3.6.2.1.	Hydroxyl groups	20
3.6.2.2.	<i>O</i> -Methylation	21

CHAPTER 4. STILBENES

4.1	Introduction	23
4.2	Structures and distribution	24
4.2.1.	Monomeric stilbenes	24
4.2.2.	Stilbene oligomers	25
4.3.	Biosynthesis of stilbenes	26
4.4.	Stilbene monomer synthesis	27
4.5.	Stilbene oligomer synthesis	29

DISCUSSION

CHAPTER 5.

5.	Monomers from <i>Pericopsis elata</i>	30
5.1.	Introduction	30
5.2	Flavanones	31
5.2.1	5,7,4'-Triacetoxyisoflavone (67)	31
5.2.2.	5,7,3',4'-Tetramethoxyflavanone (68)	32
5.3.	Isoflavones	34
5.3.1	5,7,4'-Triacetoxyisoflavone (69)	34
5.3.2.	5,7-Diacetoxy-4'-methoxyisoflavone (70)	35
5.4.	Dihydrochalcones	36
5.4.1	<i>R</i> - α -Acetoxy-4,2',4'-triacetoxydihydrochalcone (71)	36
5.5.	α -Methyldeoxybenzoins	37
5.5.1.	4'-Acetoxy-2'-hydroxy-4-methoxy- α -methyldeoxybenzoin (72)	38

5.6	Stilbenes	39
5.6.1	Monomeric stilbenes	39
5.6.1.1	<i>trans</i> -4,3',5'-Triacetoxystilbene (73)	39
5.6.1.2.	<i>trans</i> -3,4,3',5'-Tetraacetoxystilbene (74) and <i>trans</i> -3,4,3',5'-tetrahydroxystilbene (48)	40
5.6.1.3.	<i>trans</i> -2,3',5'-Triacetoxy-4-methoxystilbene (75)	42

CHAPTER 6.

DIMERIC STILBENES

6.1.	Dimeric stilbenes	44
6.6.1.	Introduction	44
6.2.	Dimeric stilbenes with a benzodioxane nucleus	47
6.2.1.	<i>rel</i> -2,3- <i>trans</i> -2-(3,4-Dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-6-[2-(3,5-dimethoxyphenyl)- <i>E</i> -1-ethenyl]benzodioxane (76)	47
6.3.	Dimeric stilbenes with the dihydrobenzofuran nucleus	51
6.3.1.	<i>rel</i> -2,3- <i>trans</i> -2-(3,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-4-[2-(3,4-dimethoxyphenyl)- <i>E</i> -1-ethenyl]-6-methoxy-2,3-dihydrobenzofuran (77)	52
6.3.2.	<i>rel</i> -2,3- <i>trans</i> -2-(3,4-Diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-4-[2-(3,4-diacetoxyphenyl)- <i>E</i> -1-ethenyl]-6-acetoxy-2,3-dihydrobenzofuran (78)	56
6.3.3.	<i>rel</i> -2,3- <i>trans</i> -2-(3,4-Dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-4-[2-(3,5-dimethoxyphenyl)- <i>Z</i> -1-ethenyl]-6-methoxy-2,3-dihydrobenzofuran (79)	60
6.3.4.	<i>rel</i> -2,3- <i>trans</i> -4-Formyl-2-(3,4-dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran (80)	63
6.3.5.	<i>rel</i> -2,3- <i>trans</i> -2-(3,5-Diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-6-[2-(3,5-diacetoxyphenyl)- <i>E</i> -1-ethenyl]-4-acetoxy-2,3-dihydrobenzofuran (81)	65

CHAPTER 7. SYNTHESIS

7.1	Introduction	69
7.2.	Synthesis of the stilbene monomer 48	70
7.3.	Synthesis of the stilbene dimers (88 and 89)	72

CHAPTER 8. Standard experimental techniques

8.1.	Chromatographic techniques	76
8.1.1.	Paper chromatography	76
8.1.2.	Column chromatography	76
8.1.3.	Thin layer chromatography	77
8.2.	Spraying reagents	77
8.2.1.	Vanillin-sulphuric acid	77
8.2.2.	Anisaldehyde	77
8.2.3.	Formaldehyde-sulphuric acid	78
8.2.4.	Bis-diazotized benzidine	78
8.3.	Chemical methods	78
8.3.1.	Acetylation	78
8.3.2.	Methylation with diazomethane	78
8.4.	Anhydrous solvents and reagents	79
8.5.	Freeze-drying	79
8.6.	Spectroscopical methods	79
8.6.1.	Nuclear magnetic resonance spectroscopy	79
8.6.2.	Circular dichroism	80
8.6.3.	Mass spectrometry	80
8.7.	Abbreviations	81

CHAPTER 9. Isolation of compounds from *Pericopsis elata*

9.1.	Enrichment of the heartwood extract	82
9.2.	Isolation of compounds from the acetone extract	82
9.2.1.	4'-Acetoxy-2'-hydroxy-4-methoxy- α -methyldeoxybenzoin (72)	83

9.2.2.	<i>R</i> - α -Acetoxy-4,2',4'-triacetoxydihydrochalcone (71)	84
9.2.3.	<i>trans</i> -4,3',5'-Triacetoxystilbene (73)	84
9.2.4.	5,7,4'-Triacetoxylisoflavone (69)	84
9.2.5.	5,7-Diacetoxy-4'-methoxylisoflavone (70)	84
9.2.6.	5,7,4'-Triacetoxylavanone (67)	85
9.2.7.	<i>trans</i> -4,3',5'-Triacetoxy-3-methoxystilbene (75)	85
9.2.8.	<i>rel</i> -2,3- <i>trans</i> -2-(3,5-Diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-6-[2-(3,5-diacetoxyphenyl)]- <i>E</i> -1-ethenyl]-4-acetoxy-2,3-dihydrobenzofuran (81)	85
9.2.9.	<i>rel</i> -2,3- <i>trans</i> -2-(3,4-Diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-4-[2-(3,4-diacetoxyphenyl)]- <i>E</i> -1-ethenyl]-6-acetoxy-2,3-dihydrobenzofuran (78)	85
9.3.	Isolation of compounds from the methanol extract	86
9.3.1.	<i>trans</i> -3,4,3',5'-Tetrahydroxystilbene (48)	86
9.3.2.	<i>trans</i> -3,4,3',5'-Tetraacetoxystilbene (74)	87
9.3.3.	<i>rel</i> -2,3- <i>trans</i> -2-(3,4-dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-4-[2-(3,5-dimethoxyphenyl)]- <i>Z</i> -1-ethenyl]-6-methoxy-2,3-dihydrobenzofuran (79)	87
9.3.4.	<i>rel</i> -2,3- <i>trans</i> -2-(3,4-Dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-6-[2-(3,5-dimethoxyphenyl)- <i>E</i> -1-ethenyl]benzodioxane (76)	88
9.3.5.	<i>rel</i> -2,3- <i>trans</i> -2-(3,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-4-[2-(3,4- dimethoxyphenyl)]- <i>E</i> -1-ethenyl]-6-methoxy-2,3-dihydrobenzofuran (77)	88
9.3.6.	<i>rel</i> -2,3- <i>trans</i> -4-Formyl-2-(3,4-dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)- 7-methoxy-2,3-dihydrobenzofuran (80)	88

CHAPTER 10. Syntheses of the monomeric and oligomeric stilbenes

10.1.	Formation of the phosphonate salt	89
10.1.1.	3,5-Dimethoxybenzyl alcohol (83)	89
10.1.2.	3,5-Dimethoxybenzyl bromide (84)	89
10.1.3	3,5-Dimethoxy-diphenyl-benzylphosphonate (salt) (85)	90
10.2.	Synthesis of the monomeric stilbene (87)	90
10.2.1.	Demethylation of the monomeric stilbene	90

10.3.	Synthesis of dimeric stilbenes (88 and 89)	90
10.3.1.	Acetylation and methylation of dimeric stilbenes (88 and 89)	91
10.3.1.	Methylation of 88	91
10.3.2.	Acetylation of 89	91

Physical data

NMR Plates 1-21

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CHAPTER 1

1. INTRODUCTION

Pericopsis elata Meeuwen (also known as *Afromorsia elata* Harms) is an economically important timber-producing species native to the Guinean equatorial forests of West and Central Africa. It has a disjunct distribution with several isolated sub-populations occurring in Cote d'Ivoire, Ghana, Central African Republic (CAR), eastern Cameroon and Congo, Democratic Republic of Congo (DRC) and Nigeria. The species is considered an excellent alternative to teak.

Pericopsis elata is a semi-gregarious species with a limited distribution. The species occurs in dryer parts of moist semi-deciduous forests with annual rainfall of 1000 – 1500 mm. *Pericopsis elata* is a tree that reaches a height of around 50 m. The trunk is buttressed to about 2.5 m then fluted, and it has a maximum diameter of approximately 2 m.



Figure 1.1. *Pericopsis elata*

Ripe, indehiscent pods, which may be dispersed in strong winds, are produced at the beginning of the dry season (August-November).¹ Each pod contains between 1-3 flat seeds. Years of abundant seed generation have been recorded but in many fruiting years, germination is said to be poor. Seedlings are reported to be drought tolerant and saplings tend to have a spreading, bushy habit. In suitable conditions, growth may be rapid, up to 1 cm increment in diameter per year. The heartwood is durable and highly resistant to termite attack. It has white-yellowish sapwood, sharply different from duramen, which is olive-brown and the sapwood is permeable.

The Democratic Republic of Congo (DRC) has the largest remaining stocks of *Pericopsis elata*. Reported threats to *Pericopsis elata* are the use of the wood by local people for charcoal production. The wood of this species is used for the construction of fine boats, decorative veneers, furniture and also works easily and takes good finish when polished. The wood is also used in external application because of its weather resistance.

The bark of *Pericopsis elata* is used by the local population for cancer treatment. Previous phytochemical studies of *Pericopsis elata* revealed the presence of monomeric stilbenes (resveratrol, piceatannol and isorhapontigenin). The recently reported interesting biological activities of stilbenes and their derivatives, such as induction of apoptosis in colon cancer and blood sugar reduction implicated the importance of plants containing stilbenoids as resources for the development of new drugs.² The revealed phytochemical studies of *Pericopsis elata* and biological activities of the stilbenes prompted us to conduct an in-depth investigation of the heartwood specie. In these study we report the isolation of both monomeric and dimeric stilbenes along with the known flavonoids.

The taxonomic classification of *Pericopsis elata* is the following

Family: *Leguminosae (Fabaceae)*
Subfamily: *Lotoideae (Papilionoideae)*
Genus: *Pericopsis*
Species: *P. elata*

¹ W. D. Hawthorne. *Oxford Forestry Institute*, **1995**, 345.

² I. Iliya, Z. Ali, T. Tanaka, M. Iinuma, M. Furusawa, K-I. Nakaya, J. Murata, D. Darnaedi, N. Matsuura and Makoto Ubukata *Phytochemistry*, **2003**, 62, 601.

2. PHENOLIC COMPOUNDS

2.1. Introduction

With the exception of proteins, lipids, carbohydrates and nucleic acids, plant chemical constituents plants are classified as either primary or secondary metabolites.^{3,4} Secondary metabolites are compounds biosynthetically derived from primary metabolites, with limited distribution in the plant kingdom, and are usually restricted to particular taxonomic groups (species, genus, family or closely related groups of families).⁵ Phenolic compounds constitute an important portion of the secondary plant metabolites.⁶ Although plant phenolics have no apparent function in a plant's primary metabolism, they often have an ecological role, that is, they are pollinator attractants, represent chemical adaptations to environmental stresses, serve as chemical defenses against microorganisms, insects and higher predators, or even other plants (allelochemicals).⁵ Phenolics in dead plant material may persist for weeks or months, which may affect decomposition organisms and processes in soils,⁷ and therefore, the functioning of the ecosystem.⁷

Phenolic compounds may have both beneficial and toxic effects on human health.^{8,9} Numerous physiological and biochemical processes in the human body produce oxygen-centered free radicals and other reactive oxygen species as by-products.¹⁰ Over-production of such free radicals can cause oxidative damage to biomolecules (for example lipids, proteins, DNA) and as a result, lipid peroxidation may take place with progressive loss in membrane fluidity, reduction in membrane potential, increase in membrane permeability to ions and finally cell death.¹¹ Increase in oxidative stress has been regarded as an important underlying factor for a number of human

³ T. A. Geissman and D. H. G. Grout. *Organic Chemistry of Secondary Plant Metabolism*, Freeman, San Francisco, **1969**.

⁴ J. Mann. (eds. E. A. Bell and B.V. Charlwood. Oxford Univ. Press, Oxford, 1978), *Encyclopedia of Plant Physiology* (Springer-Verlag, New York, **1980**, 2.

⁵ M. F. Balandrin, J. A. Klocke, E. S. Wurtele and W. H. Bollinger. *Science*, **1985**, 228, 1154.

⁶ D. Otto, S. M. Matthias, J. Schlatter and P. Frischknecht. *Environ. Health Perspect.*, **1999**, 1, 107.

⁷ J. D. Horner, J. R. Gosz and R. G. Gates. *Am. Nat.*, **1988**, 132, 869.

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⁹ J. Schalatter and J. Luethy. *Mitt geb Lebensmittelunters Hyg.*, **1986**, 77, 404.

¹⁰ M. Wink. *Phytochemistry*, **2003**, 64, 3.

¹¹ C. Yizhong, L. Qiong, S. Mei and C. Harold. *Life Sciences*, **2004**, 74, 2157.

health problems leading to many chronic diseases, such as arteriosclerosis, cancer, diabetes, aging, and other degenerative diseases in humans.¹² Plants may contain a wide variety of free radical scavenging molecules, such as phenolic compounds, which include phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes and tannins that are rich in antioxidant activity.^{13,14} Antioxidant activity is a fundamental property important for human life.¹² Many of the biological functions, including antimutagenicity, anticarcinogenicity and antiaging, among others, may originate from it.^{15,16} The intake of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing.^{15,17}

Most species of condiments, teas and other beverages such as coffee and cocoa owe their individual properties including flavors and aromas to the presence of active secondary plant phenolics such as vanillin, ephedrine and caffeine.⁵ Some of these biologically active plant phenolics have found application as drug entities or as a model compounds for drug syntheses and semi-syntheses.^{18,19} Examples include etoposide, a semi-synthetic antineoplastic agent derived from the mayapple (*Podophyllum peltatum*), which is reported to be useful in the chemotherapeutic treatment of refractory testicular carcinomas, small cell carcinomas, nonlymphetic leukemia's and non-Hodgkin's lymphomas.²⁰ Atracurium besylate, a skeletal muscle relaxant, is another new plant-based drug approved for use, which is structurally and pharmacologically related to the curare alkaloids.²¹

Due to their immobility, plants are easily attacked by snails, insects or vertebrate herbivores, bacteria, fungi and viruses.¹¹ However, they have evolved defense chemicals to ward off, inhibit or kill enemies by production of allelochemicals. Allelochemicals are substances produced by higher plants that selectively inhibit the growth of soil microorganisms or other plants or both.⁵

¹² H. E. Poulson, H. Prieme and S. Loft. *Eur. J. Cancer Prevention*, **1998**, 7, 9.

¹³ W. Zheng and S. Y. Wang. *J. Agric. Food Chem.*, **2001**, 49, 5165.

¹⁴ Y. Z. Cai, M. Sun and H. Corke. *J. Agric. Food Chem.*, **2003**, 8, 2288.

¹⁵ E. Niki. (eds. H. Ohgashi, T. Osawa, J. Terao, S. Watanabe and T. Yoshikawa), *Food Factors for Cancer Prevention*, Springer, Tokyo, **1997**, 55.

¹⁶ C. S. Yang, J. M. Landau, M. T. Huang and H. L. Newmark. *Annu. Rev. Nutri.*, **2001**, 21, 381.

¹⁷ J. Sun, Y. F. Chu, X. Z. Wu and R. H. Liu. *J. Agric. Food Chem.*, **2002**, 50, 7449.

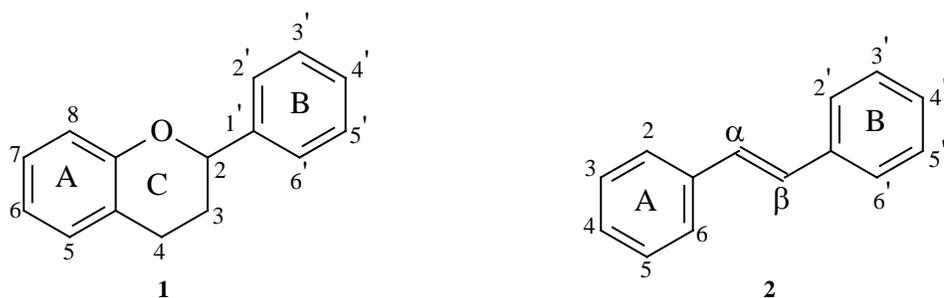
¹⁸ V. E. Tyler, L. R. Brady and J. E. Robbers. *Pharmacognosy*, (eds. Lea and Febiger, Philadelphia), **1981**, 195.

¹⁹ E. B. Roche. in *Am. Pharm. Assoc.*, **1977**, 375.

²⁰ D. A Hussar. *Am. Pharm.*, **1984**, 3, 31.

Allelopathic agents encompass a wide array of chemical types including volatile terpenoids, phenylpropanoids, quinones, coumarins, flavonoids, tannins and other phenolics and cyanogenic glycosides.⁵ These phytotoxic compounds play a role in chemical warfare between plants (allelopathic interactions) and include natural herbicides, phytoalexins (microbial inhibitors) and inhibitors of seed germination.⁵ Although many allelochemicals are strictly defense substances, others are offensive compounds that act directly in weed aggressiveness, competition and regulation of plant density.⁵

Over 8,000 phenolic structures that have been identified vary structurally from being simple molecules to highly polymerized compounds.²¹ More than ten classes of phenolic compounds have been defined on the basis of chemical structure.²² Plant phenolics are classified according to different classes such as, flavonoids (**1**) and stilbenes (**2**).



²¹ P. M. Kris –Etherton, K. D. K. Hecker, A. Bonanome, S. M. Coval, A. E. Binkoski, K. F. Hilpert, A. E. Griel and T. D. Etherton. *Am. J. Med.*, **2002**, 9, 71.

CHAPTER 3

3. FLAVONOIDS

3.1 Introduction

Flavonoids are a broad class of low molecular weight, secondary plant phenolics characterized by the flavan nucleus (1).²² Widely distributed in the leaves, seeds, bark and flowers of plants, over 6500 flavonoids have been identified to date.²³ In plants, flavonoids provide protection against ultraviolet radiation, pathogens and herbivores.²⁴ The anthocyanin co-pigments in flowers attract pollinating insects²⁵ and are responsible for the characteristic red and blue colors of berries, wines, and certain vegetables which are the major sources of flavonoids in the human diet.^{25,26,27,28,29}

Most of the beneficial health effects of flavonoids are attributed to their antioxidant and chelating abilities.²³ By virtue of their capacity to inhibit low-density lipoprotein LDL oxidation, flavonoids have demonstrated unique cardioprotective effects.^{30,31} Studies on flavonoid-rich diets revealed lower mortality from coronary heart disease, lower incidence of myocardial infarction in men³² and reduced risk of coronary heart disease by 38% in postmenopausal women.³³

Reactive oxygen species (ROS) are capable of oxidizing cellular proteins, nucleic acids and lipids.²³ Lipid peroxidation is a free-radical mediated propagation of oxidative damage to polyunsaturated fatty acids involving several types of free radicals, and termination occurs

²² K. E. Heim, A. R. Tagliaferro and D. J. Bobilya. *J. Nutri. Biochem.*, **2002**, *13*, 572.

²³ J. B. Harbone and H. Baxter. *Handbook of Natural Flavonoids*, John Wiley and Sons, Chichester, **1999**, 2.

²⁴ J. B. Harbone and C. A. Williams. *Phytochemistry*, **2000**, *55*, 481.

²⁵ J. F. Hammerstone, S. A. Lazarus and H. H. Schmitz. *J. Nutri.*, **2000**, *130*, 2086S.

²⁶ S. Caranado, P. L. Teissedre, L. Pascual-Martnez and J. C. Cabanis. *J. Agric. Food Chem.*, **1999**, *47*, 4161.

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²⁸ Y. Hara, S. J. Luo, R.L. Wickremasinha and T. Yamanishi. *Food Rev. Int.*, **1995**, *11*, 371.

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³⁰ S. Kreft, M. Knapp and I. Kreft. *J. Agric. Food Chem.*, **1999**, *47*, 4646.

³¹ A. Mazur, D. Bayle, C. Lab, E. Rock and Y. Rayssiguier. *Atherosclerosis*, **1999**, *145*, 627.

³² M. G. I. Hertog, E. J. M. Feskens, P. C. H. Hollman, M. B. Katan and D. Kromhout. *Lancet*, **1993**, *342*, 1007.

³³ L. Yochum, L. H. Kushi, K. Meyer and A. R. Folsom. *Am. J. Epidemiol.*, **1999**, *149*, 943.

through enzymatic means or by free radical scavenging of antioxidants.³⁴ ROS contribute to cellular aging,³⁵ mutagenesis,³⁶ carcinogenesis,³⁷ and coronary heart disease,³⁸ through destabilization of membranes,³⁹ DNA damage³⁸ and LDL oxidation.²³ The protective effects of flavonoids in biological systems are ascribed to their capacity to transfer electron free radicals, chelate metal catalysts,⁴⁰ activate antioxidant enzymes,⁴¹ reduce alpha-tocopherol radicals,⁴² and inhibit oxidases.⁴³

3.2. Structure and classes of flavonoids

3.2.1. Introduction

Flavonoids have a basic C₆.C₃.C₆ skeleton in common, consisting of two aromatic rings (A and B), and a heterocyclic ring (C) containing one oxygen atom (**1**). In 1953, Birch and Donovan⁴⁴ suggested that the flavonoid compounds originate from a cinnamic acid and three acetate units to give a tri-oxo acid intermediate. Tracer experiments confirmed Birch's hypothesis.⁴⁵ Shikimate was shown to contribute *via* phenylalanine and cinnamate to rings B and C (**1**), and the A-ring was found to be formally derived from three acetate units by head-to-tail condensation. According to the oxidation level of the central heterocyclic C-ring, flavonoids are grouped into different structural classes (the major ones are shown in **Figure 3.1**)

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³⁵ J. Sastre, F. V. Pallardo and J. Vina. *IUBMB life*, **2000**, 49,427.

³⁶ W. Takabe, E. Niki, K. Uchida, S. Yamada, K. Satoh and N. Noguchi. *Carcinogenesis*, **2001**, 22,935.

³⁷ S. Kawanishi, Y. Hiraku and S. Oikawa. *Mutat. Res.*, **2001**, 488, 935.

³⁸ M. A. Khan and A. Baseer. *J. Pak. Med. Assoc.*, **2000**, 50, 261.

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⁴⁴ A. J. Birch and F. W. Donovan. *Aust. J. Chem.*, **1953**, 6, 360.

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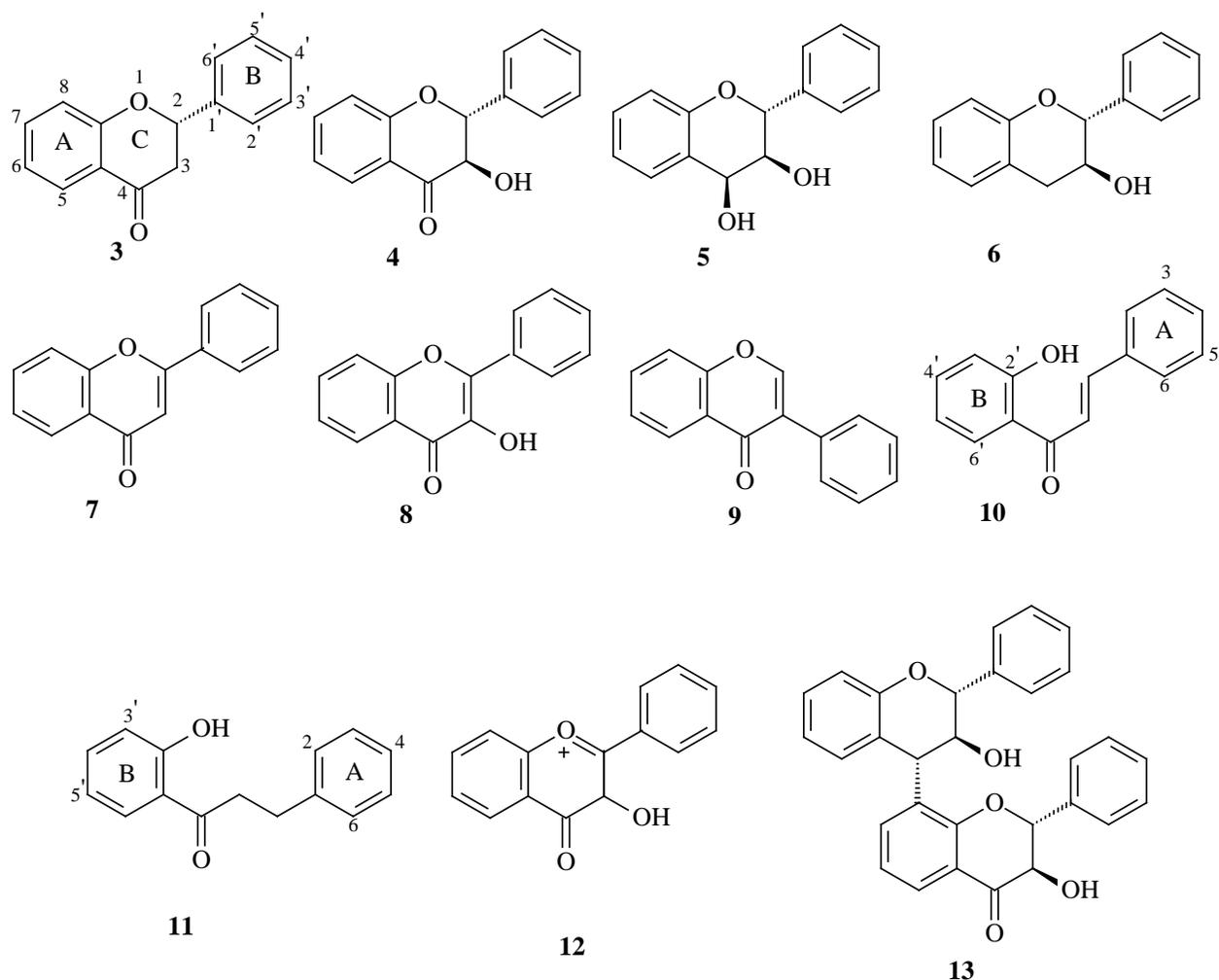


Figure 3.1: Skeleton structures of flavonoids

The principle flavonoid is the flavanone (**3**). Hydroxylation of (**3**) in position 3 leads to dihydroflavonol (**4**), which following reduction of the carbonyl group in position 4 then gives flavan-3,4-diol (**5**), and flavan-3-ol (**6**). Oxidation to a 2,3-double bond in (**3**) leads to flavones (**7**) and flavonols (**8**). Chalcones (**10** and **11**) lack the typical flavonoid structure but they are biosynthetically the precursors of flavonoids (**Scheme 3.2** page 19). Isoflavones (**9**) are distinct from the other flavonoid classes by having the B-ring attached to position 3 of the heterocyclic C-ring. Anthocyanins (**12**) possess a conjugated C-ring system giving rise to the red or blue color of these compounds. Proanthocyanidins (**13**) are condensation products of flavan-3,4-diols with flavan-3-ols.

3.3. Flavanones and dihydrochalcones

3.3.1. Introduction

Since C-2 and C-3 of their skeleton (or C- α and C- β in case of dihydrochalcones) are hydrogenated, flavanones are also called dihydroflavanones. Flavanones and dihydrochalcones are dihydroflavonoids lacking the conjugation between the A- and B-rings. The numbering system for the flavanone (**3**) is the same as that for flavones and flavonols, whereas the numbering for the dihydrochalcones (**11**) (Figure 3.2) nucleus follows that of the chalcones (**10**). Flavanones are biogenetically closely related to chalcones, and some easily isomerize (by ring opening) into chalcones during the isolation from plants.

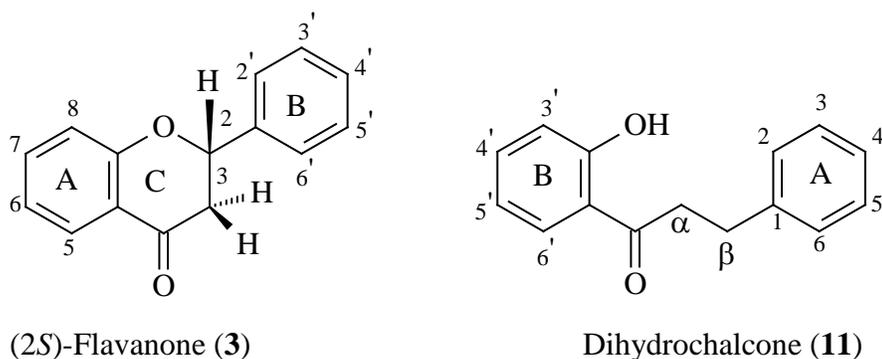


Figure 3.2: Numbering systems for flavanones and dihydrochalcones.

Since C-2 of the flavanone molecule is a stereogenic centre, two stereoisomeric forms (*S* and *R*) of each flavanone structure are possible. Most if not all naturally occurring flavanones are levorotary and belong to the (2*S*) series.⁴⁶ Dihydrochalcones do not have a stereogenic centre and are therefore achiral and optically inactive.

3.3.2. Structures of flavanones and dihydrochalcones

Within the classes of dihydroflavonoids, there is a variation in structure because of hydroxylation, methoxylation, glycosylation and alkylation of the C-atoms. At present ~319

⁴⁶ B. A. Bohm. in *The Flavonoids*, (eds. J. B. Harborne, T. J. Mabry and H. Mabry), Chapman and Hall, London, 1975, 561.

different flavanones and ~71 dihydrochalcones including glycosides are known.⁴⁷ The structures of simple naturally occurring flavanones and dihydrochalcones and their glycosides are shown in **Figures 3.3** and **3.4**, respectively.

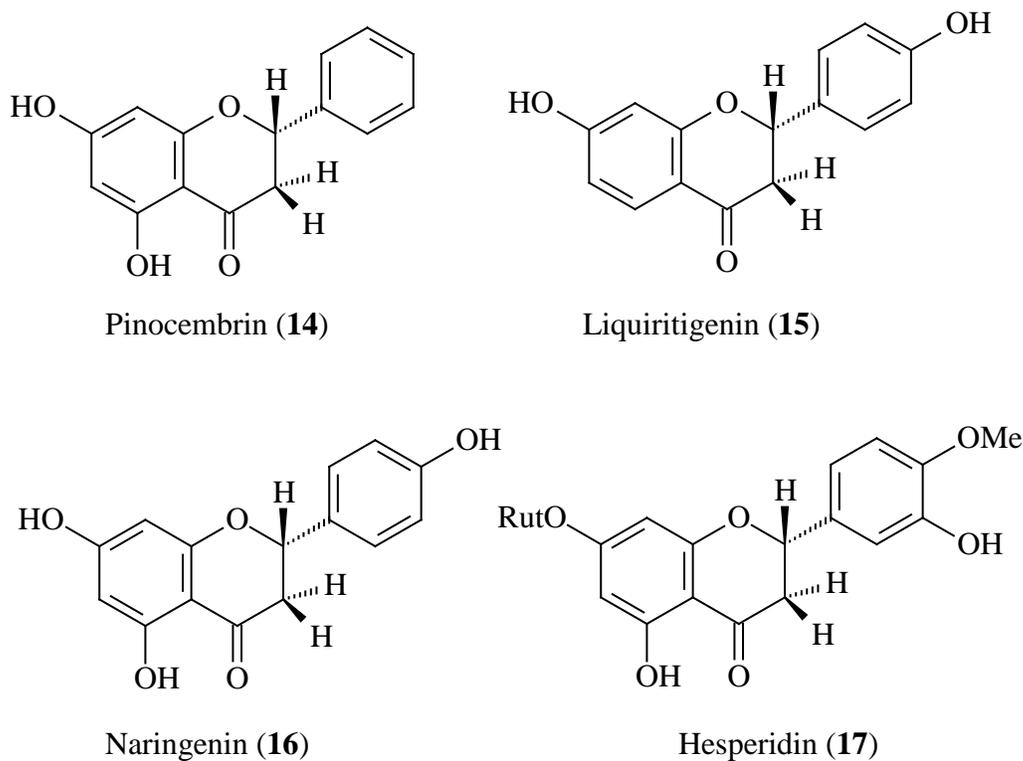


Figure 3.3. Common naturally occurring flavanones.

Rut = Rutinosyl

⁴⁷ B. A. Bohm. in *The Flavonoids*, (eds. J. B. Harborne, T. J. Mabry and H. Mabry), Chapman and Hall, London, **1988**, 329 and 583.

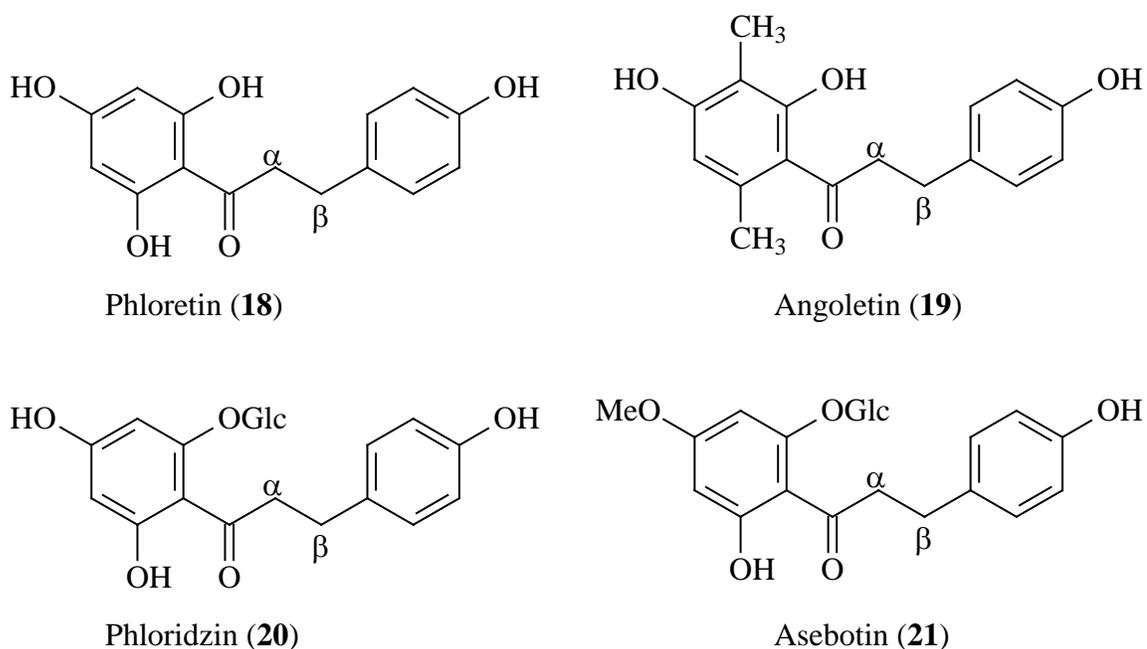


Figure 3.4: Some naturally occurring dihydrochalcones

3.3.3. Identification by spectral methods

3.3.3.1. Nuclear Magnetic Resonance Spectroscopy

Proton magnetic resonance spectroscopy has become an indispensable technique in the identification of the newly found flavonoids. It can be used to determine the oxygenation pattern of the molecule, the number of functional groups other than hydroxyls (*e.g.* *O*- and *C*-methyl and prenyl groups), to establish the number of sugars present in glycosides and to distinguish between the different classes of flavonoids.

The A- and B-ring protons of flavanones and dihydrochalcones give very similar chemical shifts, however, the NMR spectra of these two classes are easily distinguished from each other because of the differences in the signals produced by C-2 and C-3 protons in flavanones corresponding to C- β and C- α , respectively, in the dihydrochalcones (**Table 3.1**).⁴⁸ In the flavanones, the C-2 proton gives a doublet of doublets between chemical shifts δ_{H} 5.1-5.5 ppm with tetramethylsilane

⁴⁸ P. M. Dey and J. B. Harborne. *Methods in Plant Biochemistry*, **1989**, *1*, 316.

(TMS) as the internal standard in CDCl₃. The two C- α protons of dihydrochalcones produce a triplet at 3.1-3.4 ppm, and the two C- β protons a triplet at 2.8-2.9 ppm.

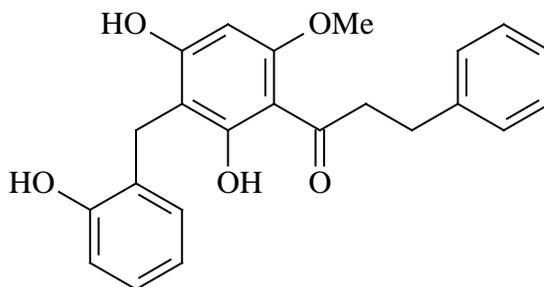
Flavonoid class	C-2/ C- β proton (ppm)	C-3/ C- α proton (ppm)
Flavanone	5.1-5.5, 1 H (dd, <i>J</i> 14.0, 3.0 Hz)	2.5-2.8, 1 H (dd, <i>J</i> 17.0, 3.0 Hz) 2.7-3.2, 1 H (dd, <i>J</i> 17.0, 14.0 Hz)
Dihydrochalcone	2.8-2.9, 2 H (t, <i>J</i> 8.5 Hz)	3.1-3.4, 2 H (t, <i>J</i> 8.5 Hz)

Table 3.1: Proton NMR chemical shifts (δ) diagnostic for the flavanone and dihydrochalcone aglycones

dd = doublet of doublets; t = triplet

3.3.4. Natural distribution

Flavanones and dihydrochalcones occur in a number of ferns and gymnosperms, in many plant families belonging to the angiosperms⁴⁹ and in a few lower plants. The dihydrochalcones and flavanones are widespread in the *Leguminosae*, *Compositae* and *Annonaceae* families. However, in the *Annonaceae* family the dihydrochalcone and the flavanone structures seem to be present only in the genera *Uvaria* and *Unona*. Flavanones and dihydrochalcones from the *Uvaria* species are especially unusual because they are C-*ortho*-hydroxybenzylated e.g. uvaretin (**22**).



Uvaretin (**22**)

Many different flavanone structures have been found in *Labiatae*, *Rutaceae* and *Roseaceae*. There are also several genera of ferns (e.g. *Pityrogramma*) and conifers (e.g. *Pinus*), which are rich in flavanones.

Dihydrochalcones have been reported from a fungus (*Phallus impudicus*), a liverwort (*Radula variabilis*), from several ferns (*Pityrogramma*, *Notholaena*, and *Adiantum* species), a conifer (*Podocarpus nubigena*) and from seventeen angiosperm families.¹ The greatest variety of structures has been found in the *Leguminosae* (in twelve genera), *Compositae* (*Helichrysum*) and *Annonaceae*. Only a small number of structures have been found in the *Ericaceae*.⁵⁰

3.3.5. Biological and pharmacological properties

Since flavonoids are phenolic compounds, they react with proteins, and thus they can react with enzymes and the biological processes in the cell.⁵⁰ This can, for example, make them toxic to certain microorganisms or animals, and inhibit their growth. Some flavanones have been shown to have inhibitory effects on microorganisms. For instance, naringenin (**16**) isolated from the wood of *Salix capraea* was active against three out of five wood-destroying fungi tested.

Dreyer and Jones⁴⁹ investigated the insect feeding deterrence of a number of flavanones and dihydrochalcones against the aphid *Schizaphis graminum*. While flavanone glycosides appeared to be inactive, flavanone aglycones showed activity. Of the dihydrochalcones tested, phloridzin (**20**) and its aglycone phloretin (**18**) showed the highest deterrence. Some dihydrochalcones appear to have uncoupling and inhibitory activities on isolated mitochondria.⁵⁰ Since phloretin (**18**) was active, and not its 2'-*O*-glucoside, phloridzin (**20**), the presence of the hydroxyl group in the 2'-position seems to be essential in this respect.

⁴⁹ D. L. Dreyer and K. C. Jones. *Phytochemistry*, **1981**, 20, 2128.

⁵⁰ P. C. de Jonge, T. Wieringa, J. P. M. van Putten, H. M. J. Kransand, K. van Dam. *Biochim. Biophys. Acta*, **1983**, 722, 219.

3.4. Isoflavones and α -methyldeoxybenzoin

Isoflavonoids (**9**) are biogenetically related to flavonoids but constitute a distinctly separate class in that they contain a rearranged C₆.C₃.C₆ skeleton, and may be regarded as derivatives of 3-phenylchroman.⁵¹ There are more than 629 known structures⁵², which may be subdivided into different classes (examples in **Figure 3.5**) according to their oxidation level and variation in the complexity of the skeleton (**9**).

The isoflavones, with ~ 234 known aglycones, form the largest part of the isoflavonoids. The four commonest isoflavones (**Figure 3.5**) are genistein (**23**), daidzein (**24**), formononetin (**25**) and biochanin A (**26**). Additional known isoflavones are derived from these basic structures by the addition of hydroxyl, methoxyl or methylenedioxy groups, for example, orobol (**29**) with 3'-ring substitution of genistein (**23**). The remaining isoflavones have isoprenyl substitution leading in many cases to extra heterocyclic rings and allyl side chains. Examples include, glyceollin (**27**) and coumestrol (**28**). Other characteristic structural features of the isoflavones include the frequent absence of a 5-hydroxyl and the presence of 6- and 2'-hydroxylation.

⁵¹ P. M. Dewick. in *The Flavonoids: Advances in research since 1986* (ed. J. B. Harborne), Chapman and Hall, London, **1993**, 117.

⁵² P. M. Dewick. in *The Flavonoids: Advances in research since 1980* (ed. J. B. Harborne), Chapman and Hall, London, **1982**, 125.

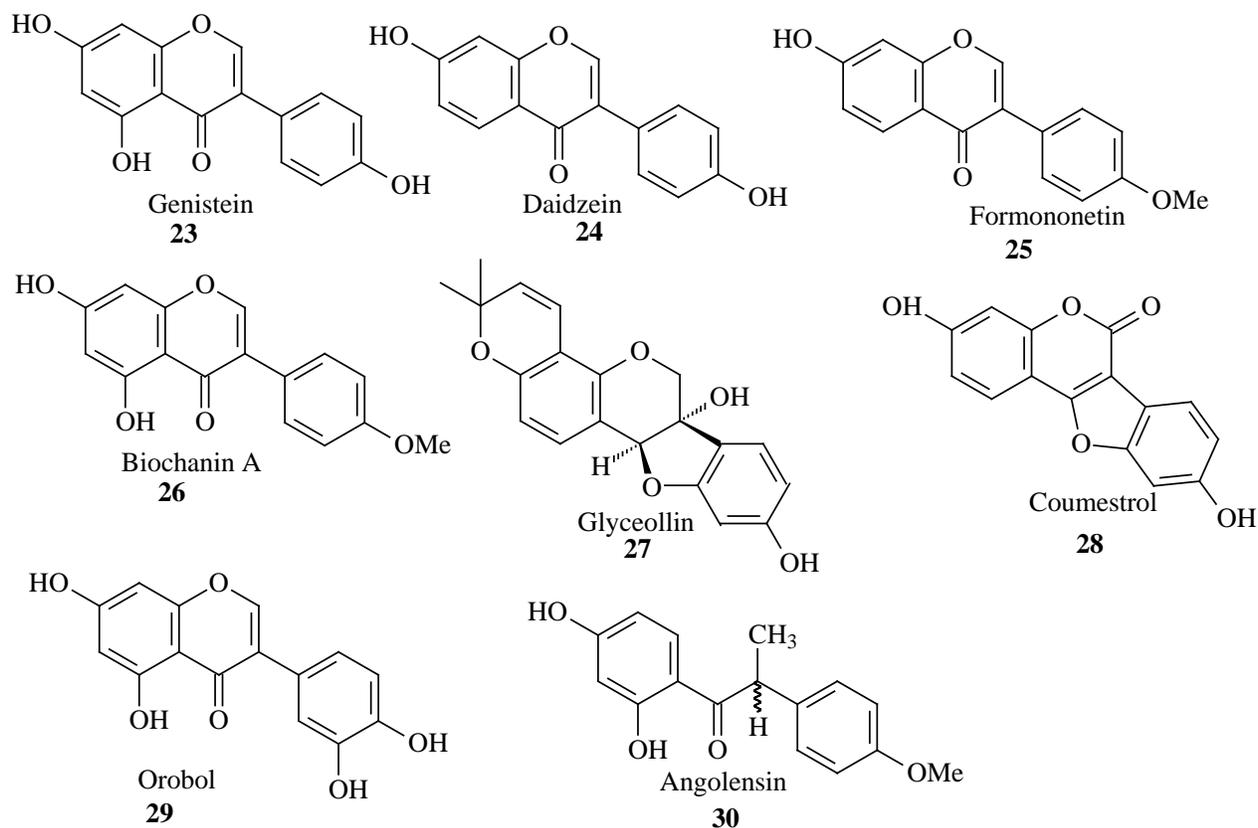


Figure 3.5. Selected examples of isoflavonoids.

Angolensin (**30**) and its methyl and cadinyl ethers are the documented members of the α -methyldeoxybenzoin class.⁵³ As these compounds co-occur with the isoflavonoids, they are probably biosynthetically closely related.

3.4.1. Characterization

3.4.1.1. Nuclear Magnetic Resonance Spectroscopy

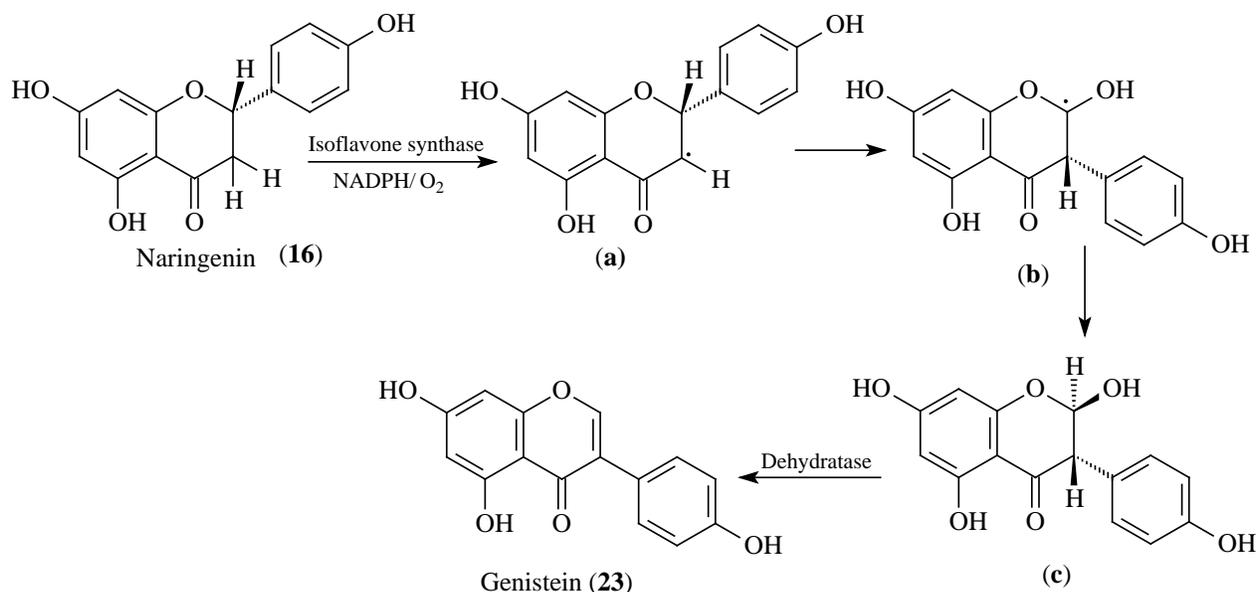
The main distinguishing feature of isoflavones proton NMR spectra from other flavonoid spectra is the resonance of a singlet H-2 proton which occurs at 7.6-8.2 ppm, (downfield from most

⁵³ J. B. Harborne. in *The Flavonoids: Advances in research since 1980* (ed. J. B. Harborne), Chapman and Hall, London, **1988**, 181.

aromatic protons). The deshielding is due to its β -relationship to the 4-carbonyl and the fact that it is on a carbon attached to the oxygen and the presence of a double bond between C-2 and C-3.

3.4.2. Natural distribution

Isoflavonoids have limited distribution in nature, being confined essentially to the subfamily *Papilionoideae* (*Lotoideae*) of the *Leguminosae*. They also occur rarely in other families such as the *Apocynaceae*, *Meliaceae*, *Pinaceae*, *Polygaceae*, *Compositae*, and *Myristicaceae*.⁵⁴ Several non-legume sources including the dicotyledons and microbial sources are known to produce isoflavonoids derivatives.⁵⁵ The richest source of isoflavonoids in the monocotyledons are the rhizomes of *Iris* species, but they have also been identified in leaves of *Patersonia*, another genus of *Iridaceae*. The limited taxonomic distribution of isoflavonoids is linked to the occurrence of the enzyme isoflavone synthase, which catalyzes the aryl migration ion [a two-step process (**a** \rightarrow **b** \rightarrow **c** \rightarrow **23**) involving hydroxylation/aryl migration followed by dehydration] leading to the formation of an isoflavone from a flavanone⁵⁶ (**Scheme 3.1**).



Scheme 3.1. Mechanism of isoflavone biosynthesis.

⁵⁴ S. Tahra and R. K. Ibrahim. *Phytochemistry*, **1995**, 38, 1073.

⁵⁵ M. Yamaki, T. Kato, M. Kashihara and S. Takagi. *Planta Med.*, (**1990**), 56, 335.

⁵⁶ P. M. Dewick, in *The Flavonoids: Advances in Research Since 1980* (ed. J. B Harborne), Chapman Hall, London, **1988**, 535.

3.4.3. Biological properties

Isoflavonoids show a wide range of biological properties but the three most important ones are the oestrogenic activities of simple isoflavones, the antifungal and antibacterial properties of the isoflavonoids as phytoalexins, and the insecticidal properties. Isoflavones were first discovered to have oestrogenic activity when sheep which were grazed on pastureland containing *Trifolium subterraneum* for longer periods than normal, were found to have reduced fertility. The two isoflavones, genistein (**23**) and formononetin (**25**), isolated from this clover by Bradbury and White were shown to be the active principles.⁵⁷ The oestrogenic activity is due to the ability of these isoflavones to mimic the steroidal nucleus of the natural female hormone oestrogen.

Phytoalexins are antimicrobial, usually antifungal substances, which are produced as part of the plant's natural defense system in response to fungal or bacterial invasion. Over 400 legumes have been surveyed for phytoalexins and most respond positively. The isoflavones genistein (**23**) and daidzein (**24**) have been identified in the root of legumes, where they have the ability to inhibit the nodulating ability of *Rhizobium* in the nitrogen-fixing symbiosis.⁵⁸

3.5. Biosynthesis

3.5.1. Flavanones, chalcones and isoflavonoids

All the flavonoids derive their carbon skeletons from two basic compounds, 4-coumaroyl-CoA and three molecules of malonyl-CoA.⁵⁹ The pathway leading to the flavonoid precursors and various flavonoid classes with their respective enzymes are outlined in **Scheme 3.2** which illustrates the general relationship of different types of compounds in the biosynthesis of flavonoids. The flavonoid C₆.C₃.C₆ carbon backbone is represented by the A-, B- and C-rings (**1**).

⁵⁷ R. B. Bradbury and D. E. White. *Vitamins and Hormones*, **1954**, *12*, 207.

⁵⁸ J. B. Harborne. in "*Plant Flavonoids in Biology and Medicine*" (eds. V. Cody, E. Middleton, J. B. Harborne and A. Beretz), R. Alan Liss, New York, **1988**, *2*, 17.

⁵⁹ U. Weiss and J. M. Edwards. *The Biosynthesis of Aromatic Compounds*. John Wiley & Sons, New York, **1980**, 326.

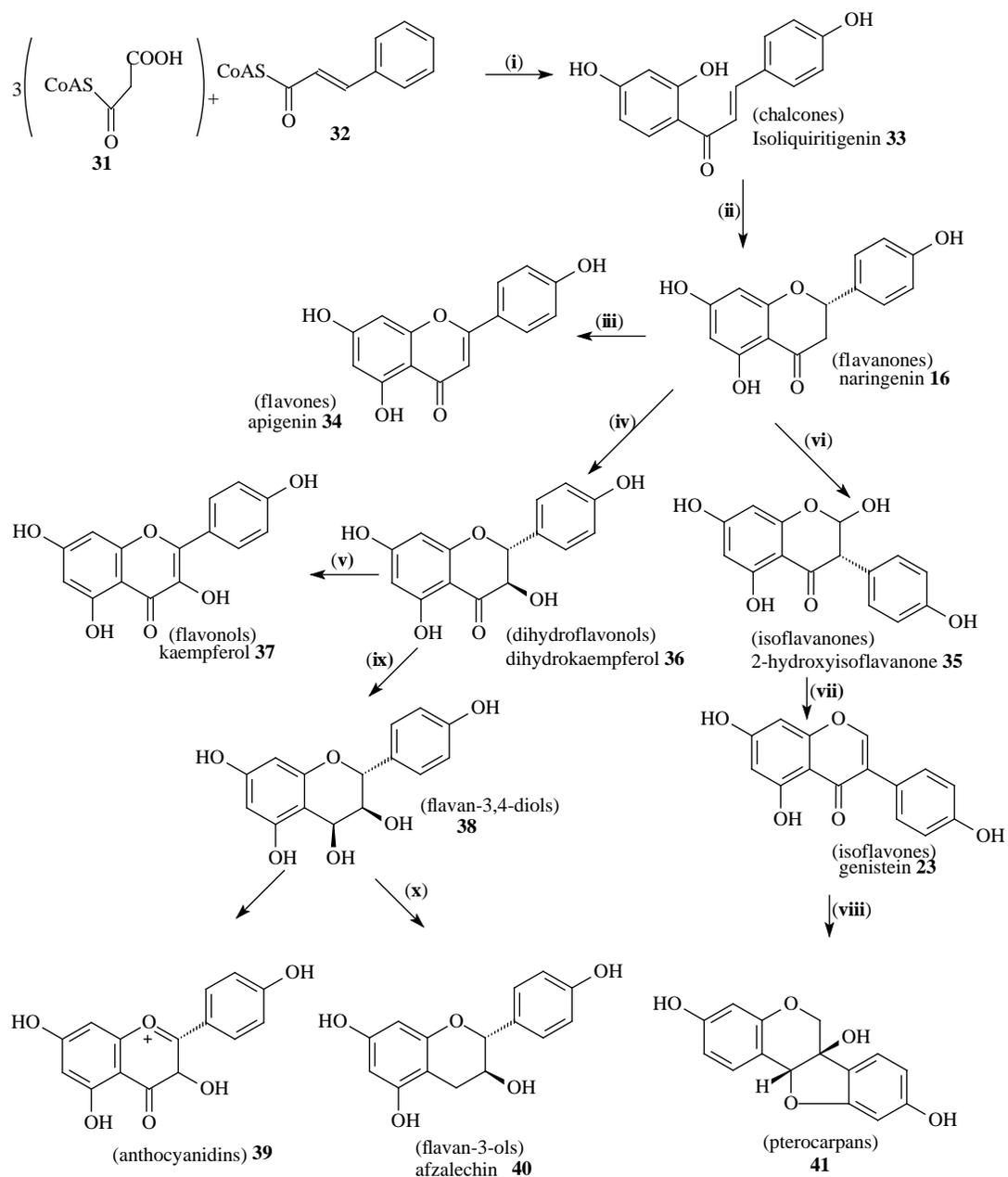
The initial formation of a flavonoid is catalyzed by chalcone synthase (CHS) (**i**), which forms the chalcone e.g. isoliquiritigenin (**33**) from malonyl-CoA (**31**) and 4-coumaroyl-CoA (**32**) in the presence of polyketide reductases (PKR) and NADPH. Because 4-coumaroyl-CoA is the principle physiological substrate for CHS, the B-ring of chalcones is primarily hydroxylated in the 4-position, and the respective flavonoids, therefore, in the 4'-position. The resulting chalcone is converted into a flavanone, e.g. naringenin (**16**) by chalcone-flavanone isomerase (CHI) (**ii**), which catalyzes the stereospecific cyclization of chalcones to (2*S*)-flavanones.

Introduction of a 2,3-double bond in flavanones leads to the abundant flavones [e.g. apigenin (**34**)]. Two types of enzymes can catalyze this reaction i.e. flavone synthase I (FNS I) (**iii**) an oxoglutarate-dependent dioxygenase, and flavone synthase II (FNS II), an NADPH-dependent cytochrome P450 species which accomplishes dehydrogenation.

The 5-deoxyflavanones are important intermediates in the formation of isoflavones, also leading to pterocarpan (**41**). The first step in isoflavone formation is catalyzed by 2-hydroxyisoflavanone synthase (IFS) (**vi**), an NADPH-dependent cytochrome P450 mixed monooxygenase. Subsequent action of a dehydratase (IFD) (**vii**) leads to the respective isoflavone [e.g. genistein (**23**)]. The pterocarpan (**41**) formation is catalyzed by pterocarpan synthase (**viii**) with 2-hydroxyisoflavanone as a substrate.

Hydroxylation of flavanones at C-3 leads to dihydroflavonols [e.g. dihydrokaempferol (**36**)]. This step is catalyzed by flavanone 3-hydroxylase (FHT) (**iv**). Dihydroflavonols are the precursors of flavonols (**37**) [e.g. kaempferol] and flavan-3, 4-diol (**38**) synthesis, the latter being the direct biosynthetic intermediate to flavan-3-ol and anthocyanidins (**39**). Flavonols are formed from dihydroflavonols by the introduction of a 2,3-double bond catalyzed by flavonol synthase (FLS) (**v**).

Reduction of the carbonyl group of the dihydroflavonols leads to flavan-3,4-diols (**38**), also called leucoanthocyanidins, which is catalyzed by dihydroflavonol 4-reductase (DFR) (**viii**) with NADPH as the reducing factor. Flavan-3-ols (**40**) are formed by further reduction of flavan-3,4-diols at C-4 by leucoanthocyanidin reductase (LAR) (**x**) in the presence of NADPH.



Scheme 3.2. Flavonoid biosynthesis

(i) Chalcone synthase

(ii) Chalcone isomerase

(iii) Flavone synthase

(iv) Flavanone-3-hydroxylase

(v) Flavanol synthase

(vi) 2-Hydroxyisoflavanone synthase

(vii) Isoflavanone dehydratase

(viii) Pterocarpan synthase

(ix) Dihydroflavonol-4-reductase

(x) Leucoanthocyanidin reductase

3.6. Chemistry of the flavonoids

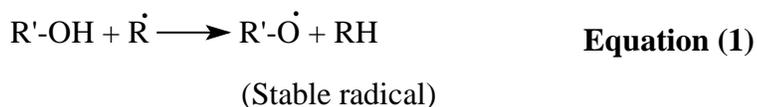
3.6.1. Chemical structure

Flavonoids are benzo- γ -pyrone derivatives (**1**) which are classified according to the substitution and the oxidation state of the C-ring. Flavonoids also differ in the arrangements of hydroxyl, methoxy, and glycosidic substituents. During metabolism, hydroxyl groups are added, methylated, sulfated or glucuronidated.²³ In food, flavonoids exist primarily as 3-*O*-glycosides and polymers,²⁶ which comprise a substantial fraction of dietary flavonoid intake.⁶⁰

3.6.2. Structural features and antioxidant activity

3.6.2.1. Hydroxyl groups

The antioxidant activity of flavonoids and their metabolites in *vitro* depends upon the arrangement of functional groups on the skeletal structure.⁴⁴ The arrangement of substituents is a greater determinant of antioxidant activity than the flavan backbone alone.²³ Free radical scavenging capacity is attributed to the high reactivity of hydroxyl substituents that participate in the reaction (**Equation 1**) to form a stable radical.



The superoxide anion radical ($\text{O}_2^{\cdot-}$) is an obligate byproduct of normal aerobic metabolism⁶¹ that is generated by one electron transfer to molecular oxygen. ($\text{O}_2^{\cdot-}$) is a precursor of multiple other and more toxic reactive oxygen species (ROS), such as the hydroxyl radical (HO^{\cdot}), hypochlorous acid (HOCl), and singlet oxygen ($^1\text{O}_2$), which is formed in the reaction with hydrogen peroxide.⁶² The B-ring hydroxyl configuration is a significant contributor to scavenging of reactive oxygen species (ROS)⁶³ and reactive nitrogen species (RNS).⁶⁴ Based upon fundamental chemical

⁶⁰ C. Santos-Benguela and A. Scalbert. *J. Food Sci. Agri.*, **2000**, 80, 1094.

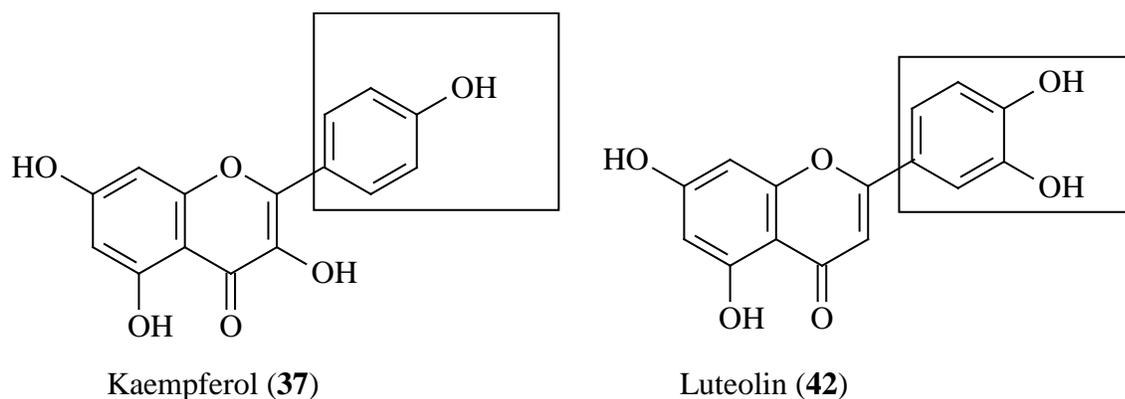
⁶¹ I. Fridovich. *The biology of Oxygen Radicals Science*, **1978**, 201, 875.

⁶² Y. Mao, L. Zang and X. Shi. *Biochem. Mol. Biol. Int.*, **1995**, 36, 227.

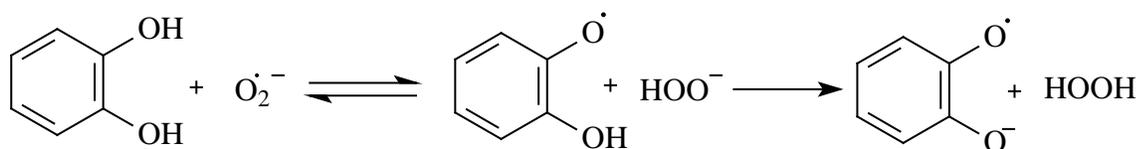
⁶³ A. S. Pannala, T. S. Chan, P. J. O'Brien and C. A. Rice-Evans. *Biochem. Biophys. Res. Commun.*, **2001**, 282, 1161.

⁶⁴ N. Kerry and C. Rice-Evans. *J. Neurochem.*, **1999**, 73, 247.

principles, the ene-diol functionality in the electron-rich aromatic B-ring system is prone to flavonoids oxidation. Hydroxyl groups on the B-ring donate a hydrogen and a electron to hydroxy, peroxy, and peroxyxynitrite radicals, stabilizing them and giving rise to a relatively stable flavonoid radical (**equation 1**). The catechol B-ring (3',4'-dihydroxy) (**Scheme 3.3**) capable of readily donating hydrogen (and electron) to stabilize a radical species, strongly enhances lipid peroxidation,⁶⁵ for example, the peroxy radical scavenging ability of luteolin (**42**) substantially exceeds kaempferol (**37**),⁶⁶ both having identical hydroxylation patterns on the A-ring, but kaempferol lacking the B-ring catechol.



Oxidation of a flavonoid occurs on the B-ring when the catechol arrangement is present, yielding a stable *ortho*-semiquinone radical through electron delocalization.⁶⁷ Flavones lacking catechol or *o*-trihydroxyl (pyrogallol) systems form relatively unstable radicals and are thus weak scavengers.⁶⁸



Scheme 3.3

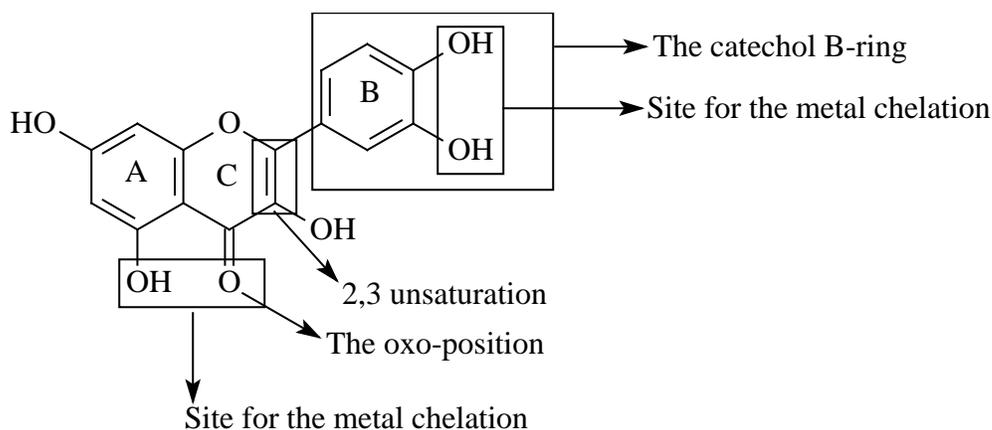
Other structural features important for antioxidant nature include the presence of 2,3 unsaturation in conjugation with a 4-oxo-function in the C-ring and the presence of functional groups capable of binding transition metal ions, such as iron and copper [for example quercetin (**43**)].

⁶⁵ A. J. Dugas, J. Castaneda-Acosta, G. C. Bonin, K. L. Price, N. H. Fischer and G. W. Winston. *J. Nat. Prod.*, **2000**, 63, 327.

⁶⁶ S. A. B. E. Van Acker, M. J. De Groot, D. J. van den Berg, M. N. J. L. Tromp, G. D. O. den Kelder, W. J. F. van der Vijgh and A. Bast. *Chem. Res. Toxicol.*, **1996**, 9, 1305.

⁶⁷ A. Arora, M. G. Nair and G. M. Srasburg. *Free Radic. Biol. Med.*, **1998**, 24, 1355.

⁶⁸ Z. Gao, K. Huang, X. Yang and H. Xu. *Biochim. Biophys. Acta*, **1999**, 472, 643.



Quercetin (**43**)

3.6.2.2. *O*-methylation

The differences in antioxidant activity between polyhydroxylated and polymethoxylated flavonoids are most likely due to differences in both hydrophobicity and molecular planarity.¹ Suppression of antioxidant activity by *O*-methylation⁶⁸ may reflect steric effects that perturb planarity. Although the ratio of methoxy to hydroxyl substituents does not necessarily predict the scavenging ability of a flavonoid, the B-ring is particularly sensitive to the position of the methoxy group.¹ Alternating a 6'-OH/4'-OMe configuration to 6'-OMe/4'-OH completely abolishes the scavenging of DPPH by inducing coplanarity.⁶⁹

⁶⁹ L. Matthiesen, K. E. Malterud and R. B. Sund. *Free Radic. Biol. Med.*, **1997**, 2, 307.

4. STILBENES

4.1. Introduction

Stilbenes (2) are a class of phenolic compounds, whose occurrence in plants has been reported^{70,71,72} mainly in grapes and wines.⁷³ These compounds have been shown to have some important biological activities,⁷⁴ act as antifungal agents,⁷⁵ and as antimicrobial inhibitors.^{76,77} In addition, they possess cyclooxygenases COX-1 and COX-2 (which are respectively constitutive and inducible enzymes that catalyze the production of pro-inflammatory prostaglandins from arachidonic acid),⁷⁸ inhibitory effects³ and anti-HIV 1 and cytotoxic effects.⁷⁹ They also affect lipid peroxidation,⁸⁰ LDL oxidation,⁸¹ arachidonate metabolism,⁸² root growth,^{83,84} antioxidant and vasodilation capacities,⁷⁴ and function as phytoalexins⁸⁴ and tyrosinase inhibitors.⁸⁵

Stilbenes play important roles in plants, especially in heartwood protection as part of both constitutive and inducible defense mechanisms, and in dormancy and growth inhibition.⁸⁶ The role of phytostilbenes and related compounds is considered to contribute to the durability of

⁷⁰ M. Cuendet, O. Potterat, A. Salvi, B. Testa and K. Hostettmann. *Phytochemistry*, **2000**, *54*, 871.

⁷¹ T. Pacher, C. Seger, D. Engelmeie, S. Vajrodaya, O. Hoferand H. Greger. *J. Nat. Prod.*, **2002**, *65*, 820.

⁷² B. N. Su, M. Cuendet, M. E. Hawthorne, L. B. S. Kardono, S. Riswan, H. H. S. Fong, R. G. Metha, J. M. Pezzuto and A. D. Kinghorn. *J. Nat. Prod.*, **2002**, *65*, 163.

⁷³ J. Burns, T. Yokota, H. Ashihara, M. E. J. Lean and A. Crozier. *J. Agric. Food Chem.*, **2002**, *50*, 337.

⁷⁴ R. L. Williams, M. Elliot, R. Perry, and B. K. Greaves. *Polyphenols Communications 96, Bordeaux Franceb*, **1996**, 210 and 489.

⁷⁵ T. P. Schultz, W. D. Boldin, T. H. Fischer, D. D. Nicholas and K. D. McMurtrey. *Phytochemistry*, **1992**, *31*, 3801.

⁷⁶ M. M. Chan. *Biochem. Pharmacol.*, **2002**, *63*, 99.

⁷⁷ J. J. Dotcherty, M. M. Fu and M. Tsai. *J. Antimicrob. Chemotherapy*, **2001**, *47*, 871.

⁷⁸ S. D. Hursting, T. J. Slaga, S. M. Fischer, J. DiGiovami and J. M. Phang. *J. Nat. Cancer Inst.*, **1999**, *91*, 215.

⁷⁹ J. R. Dai, Y. F. Hallock, J. H. Cardellina and M. R. Boyd. *J. Nat. Prod.*, **1998**, *61*, 351.

⁸⁰ E. N. Frankel, A. L. Waterhouse and J. E. Kinsella. *Lancet*, **1993**, *341*, 1004.

⁸¹ Y. Kimura, H. Okuda and S. Arichi. *Biochim. Biophys. Acta*, **1985**, *834*, 278.

⁸² H. Arichi, Y. Kimura, H. Okuda, M. Baba, M. Kozawa and S. Arichi. *Chem. Pharm. Bull.*, **1982**, *30*, 1766.

⁸³ E. H. Sieman and L. L. Creasy. *Am. J. Enol. Vitic.*, **1992**, *43*, 49.

⁸⁴ K. Shimuzi, R. Kondo and K. Sakai. *Planta Medica*, **2000**, *66*, 11.

⁸⁵ P. Torres, J. G. Avila, A. Romo de Vivar, A. M. Garcia, J. C. Marin, E. Aranda and C. L. Cespedes. *Phytochemistry*, **2003**, *64*, 463.

⁸⁶ R. Croteau, T. M. Kutchan and N. G. Lewia. *American Society of Plant Physiologists, Rockville, MD*, **2000**, 1250.

wood.⁸⁷ Formation of stilbenes occurs when tissues are dying slowly, usually due to desiccation of tissues.⁸⁸ Stilbenes are also synthesized as a secondary response against mechanical wounds, fungal infections and insects infestations, and they complement the action of resin acids which also have fungitoxic properties.⁸⁹ Certain stilbenes, besides being toxic to insects and other organisms, have mammalian and nematocidal properties.⁸⁸

4.2. Structure and distribution

4.2.1. Monomeric stilbenes

Monomeric stilbenes (**44-52**) ranging from the unsubstituted *trans*-stilbene (**2**) from *Alnus* and *Petivera* to the hexasubstituted combretastatin A-1 (**52**) from *Combretum caffrum* are more widely distributed in both gymnosperms and angiosperms.⁹⁰ Stilbenes often co-occur with flavonoids, which are related to the stilbenes on biogenetic grounds. Resveratrol (*trans*-4,3',5'-trihydroxystilbene) (**47**) is a phytoalexin, produced by plants in response to damage, particularly in vines,⁹¹ pines, and legumes⁹² and is a representative stilbene in the field of polyphenol based studies.⁹³ Resveratrol and its glucosides are widely reported to be beneficial to health. They are used in the treatment of a wide variety of diseases including dermatitis, gonorrhoea, fever, hyperlipidemia, arteriosclerosis and inflammation.^{94,95}

A common oxygenation pattern of the natural stilbenes is the 3,5-dioxy substitution, thus pinosylvin (**45**) and its monomethyl and dimethyl ethers (**46**), the first stilbenes to be isolated from wood, carry 3,5-dioxy substituents.⁹⁶ Most stilbenes isolated from natural sources have the *trans* (or *E*) configuration (**2**). However, Rowe et al⁹⁷ have isolated the *cis* (or *Z*) isomer of

⁸⁷ J. H. Hart. *Ann. Rev. Phytopathology*, **1981**, 19, 437.

⁸⁸ F. Jorgensen. *Phytochemistry*, **1961**, 7, 13.

⁸⁹ T. L. Eberhart and R. A. Young, *J. Agric. Food Chem.*, **1994**, 42, 1704.

⁹⁰ J. Gorham. *Prog. Phytochemistry*, **1980**, 17, 99.

⁹¹ P. Langcake, R. J. Pryce. *Physiol. Plant Pathol.*, **1976**, 9, 77.

⁹² G. J. Soleas, E. P. Diamandis and D. M. Goldberg. *Clin. Biochem.*, **1997**, 30, 91.

⁹³ S. Erkoc, N. Keskin and F. Erkoc. *Theochem*, **2003**, 631, 67.

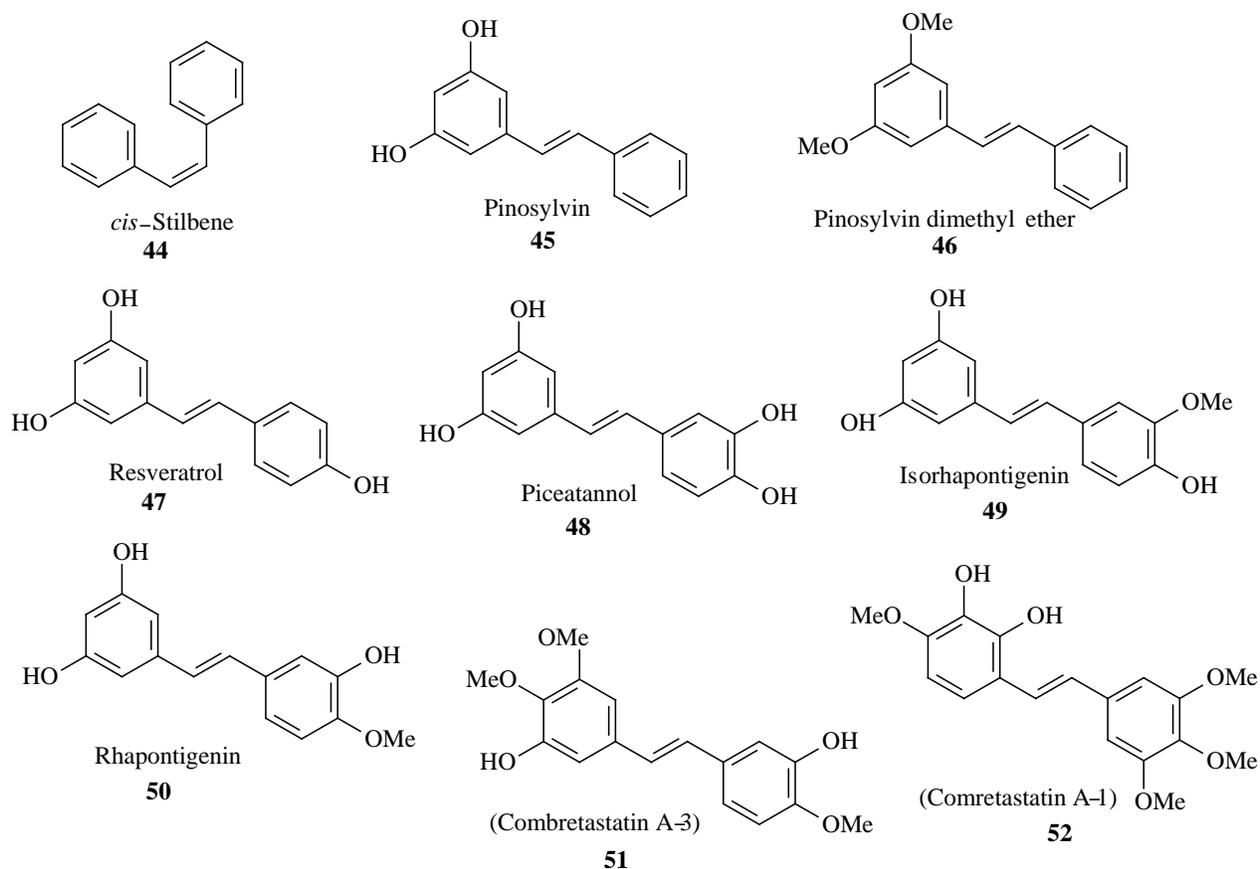
⁹⁴ H. Arichi, Y. Kimura, K. Okuda, K. Baba, K. Kozawa and S. Arichi. *Chem. Pharm. Bull.*, **1982**, 30, 1766.

⁹⁵ M. M. Chan. *Biochem. Pharmacol.*, **2002**, 63, 99.

⁹⁶ H. Erdtman. *Naturwissenschaften*, **1939**, 27, 130.

⁹⁷ J. W. Rower, C. L. Bower and E. R. Wagner. *Phytochemistry*, **1969**, 8, 235.

pinosylvin dimethyl ether from the bark of *Pinus banksiana* which contains a mixture of the *cis/trans* isomers with the *trans* isomer (**46**) dominating.



4.2.2. Stilbene oligomers

Natural stilbene oligomers (**53-57**) are a group of compounds mostly obtained from nine plant families, namely *Dipterocarpaceae*, *Vitaceae*, *Cyperaceae*, *Leguminosae*, *Gnetaceae*, *Iridaceae*, *Celastraceae*, *Paeoniaceae* and *Moraceae*.⁹⁸ Various biological activities, such as, chemoprevention of cancer,⁹⁹ protein kinase C inhibition,¹⁰⁰ anti-HIV and cytotoxicity,¹⁰¹ anti-

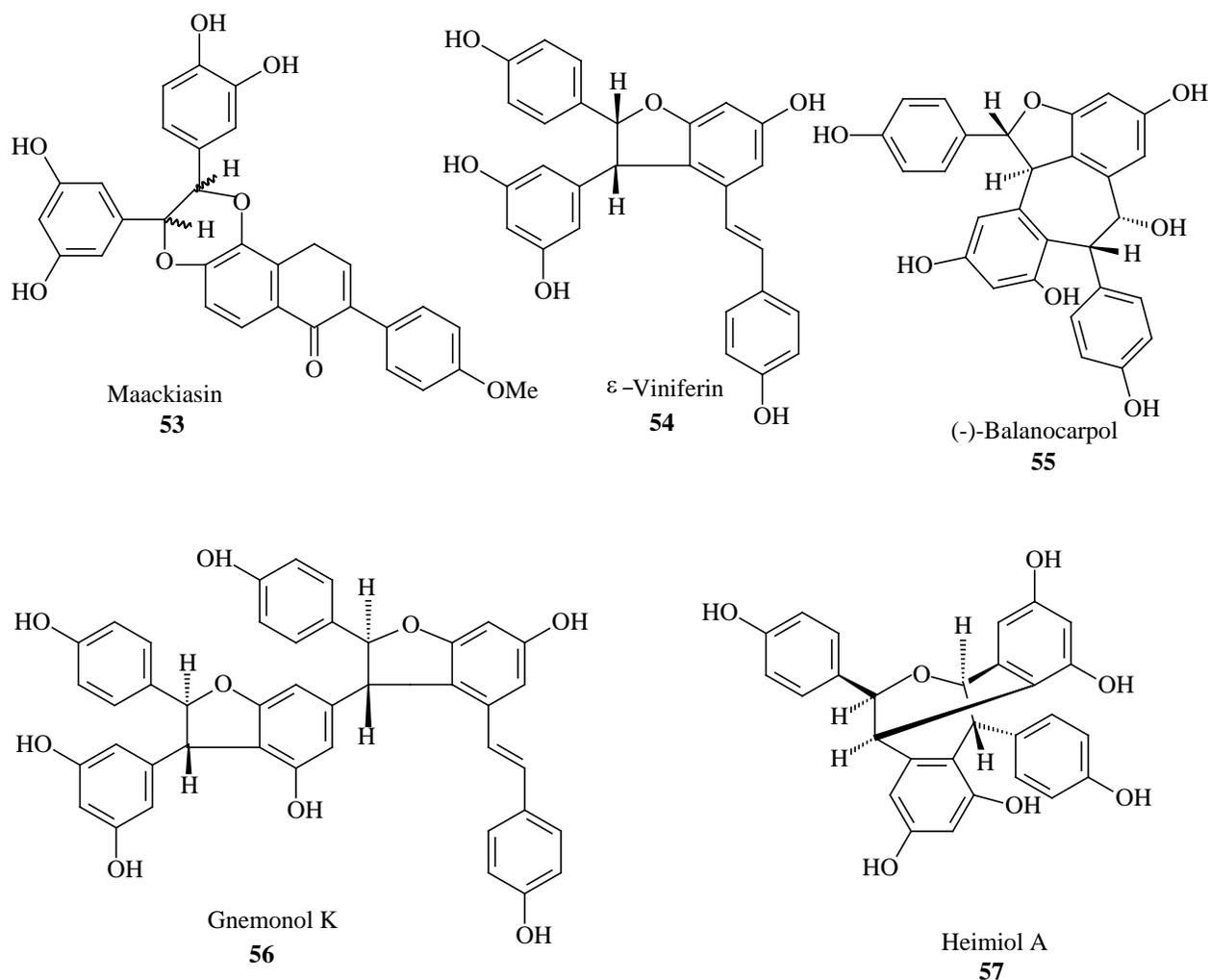
⁹⁸ N. Li, X. M. Li, K. S. Huang and M. Lin. *Acta Pharm. Sin.*, **2001**, *36*, 944.

⁹⁹ M. Jang, L. Cai, G. O. Udeani, K. V. Slowing, C. F. Thomas, C. W. W. Beecher, H. H. S. Fong, N. R. Farnsworth, A. D. Kinghorn, R. G. Mehta, R. C. Moon and J. M. Pezzuto. *Science*, **1997**, *275*, 218.

¹⁰⁰ P. Kulanthaivel, W. P. Janzen, L. M. Ballas, J. B. Jiang, C. Q. Hu, J. W. Darges, J. C. Seldin, D. J. Cofield and L. M. Adamas. *Planta Med.*, **1995**, *61*, 41.

¹⁰¹ J. R. Dai, Y. F. Hallock, J. H. Cadellina II and M. R. Boyd. *J. Nat. Prod.*, **1998**, *61*, 351.

fungal,¹⁰² and cyclooxygenase (COX I, COX II) inhibition^{103,104} have been found in stilbene oligomers. In addition, a number of natural dimeric stilbenes exhibited potent anti-inflammatory activities, including inhibition of leukotriene (LTB₄, C₄, D₄) and its receptor antagonism, affinity of HL 60, and CEC multiplet in *vitro* and in *vivo* models.^{105,105,106} Most of the oligostilbenoid polyphenols are generated from resveratrol by oxidative phenolic coupling and like resveratrol possess the typical resorcinol arrangement. Stilbene monomer building blocks with the catechol arrangement are relatively infrequent precursors in oligostilbenoid biosynthesis.



¹⁰² P. H. Ducrot, A. Kollmann, A. E. Bala, A. Majira, L. Kerhoas, R. Delorme and J. Einhorn. *Tetrahedron Lett.*, **1998**, 39, 9655.

¹⁰³ R. H. Cichewicz, S. A. Kouzi and M. T. Hamann. *J. Nat. Prod.*, **2000**, 63, 29.

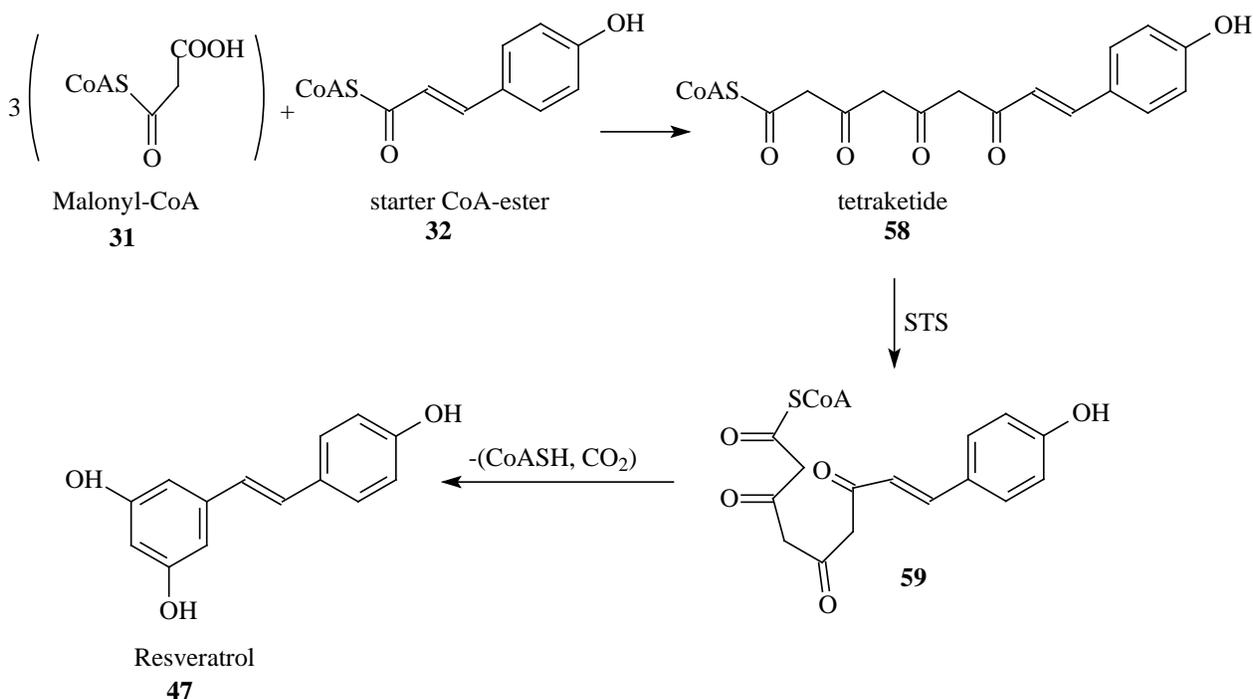
¹⁰⁴ J. Chen. Master Thesis, *Chinese Academy of Medical Sciences and Perking Union Medical College*, **1997**.

¹⁰⁵ Y. T. Li, M. Zhong, Y. J. Deng, X. Y. Zhu and G. F. Cheng. *Acta Pharm. Sin.*, **1999**, 34, 189.

¹⁰⁶ Y. N. Hu, X. Y. Zhu, X. L. Liang and G. F. Cheng. *Acta Pharm. Sin.*, **2001**, 36, 81.

4.3. Biosynthesis of stilbenes

The stilbene backbone is synthesized by the enzyme stilbene synthase (STS), which is structurally and functionally closely related to chalcone synthase (CHS). The two enzymes are polyketide synthases and therefore catalyze the linking of acyl-CoA units by repetitive condensations associated with decarboxylation. Stilbene synthase uses a starter CoA (**32**) from the phenylpropanoid pathway and performs three sequential condensation reactions with C₂ units from decarboxylated malonyl-CoA (**31**) to form a linear tetraketide intermediate (**58**), which is folded *via* (**59**) to form a new aromatic ring system (**47**). Natural stilbenes appear to be significant only to certain genera and seem to be expressive taxonomic markers.

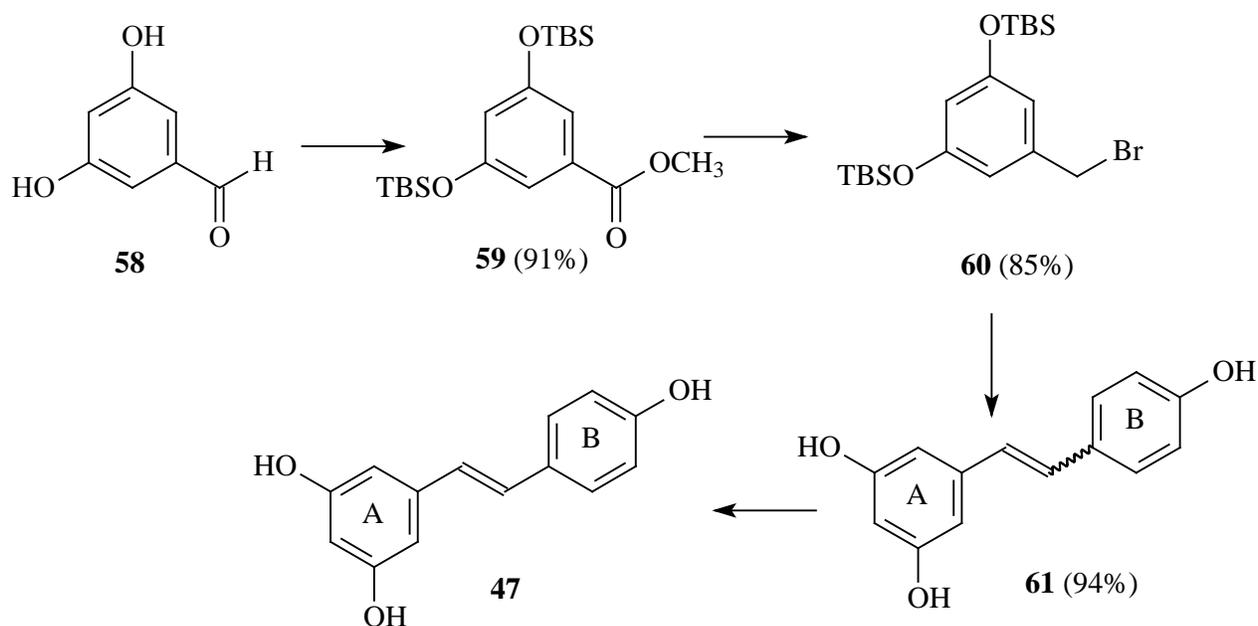


Scheme 4.1. Biosynthesis of stilbenes

4.4. Synthesis of the monomeric stilbenes

Resveratrol (**47**), (*trans*-4,3',5'-trihydroxystilbene), found in grapes and a variety of medicinal plants, is a naturally occurring phytoalexin that protects against fungal infections.^{7,8} Despite their high fat diet and heavy smoking habits, people in the south of France have a very low incidence

of coronary heart disease (CHD). This so-called *French Paradox* has strongly been related with wine consumption.¹⁰⁷ Although resveratrol has numerous biological activities in vitro, there is little produced from raw materials and as a result, synthetic procedures are needed for the formation of substantial amounts of resveratrol.¹⁰⁹



Scheme 4.2. Synthesis of stilbene monomers

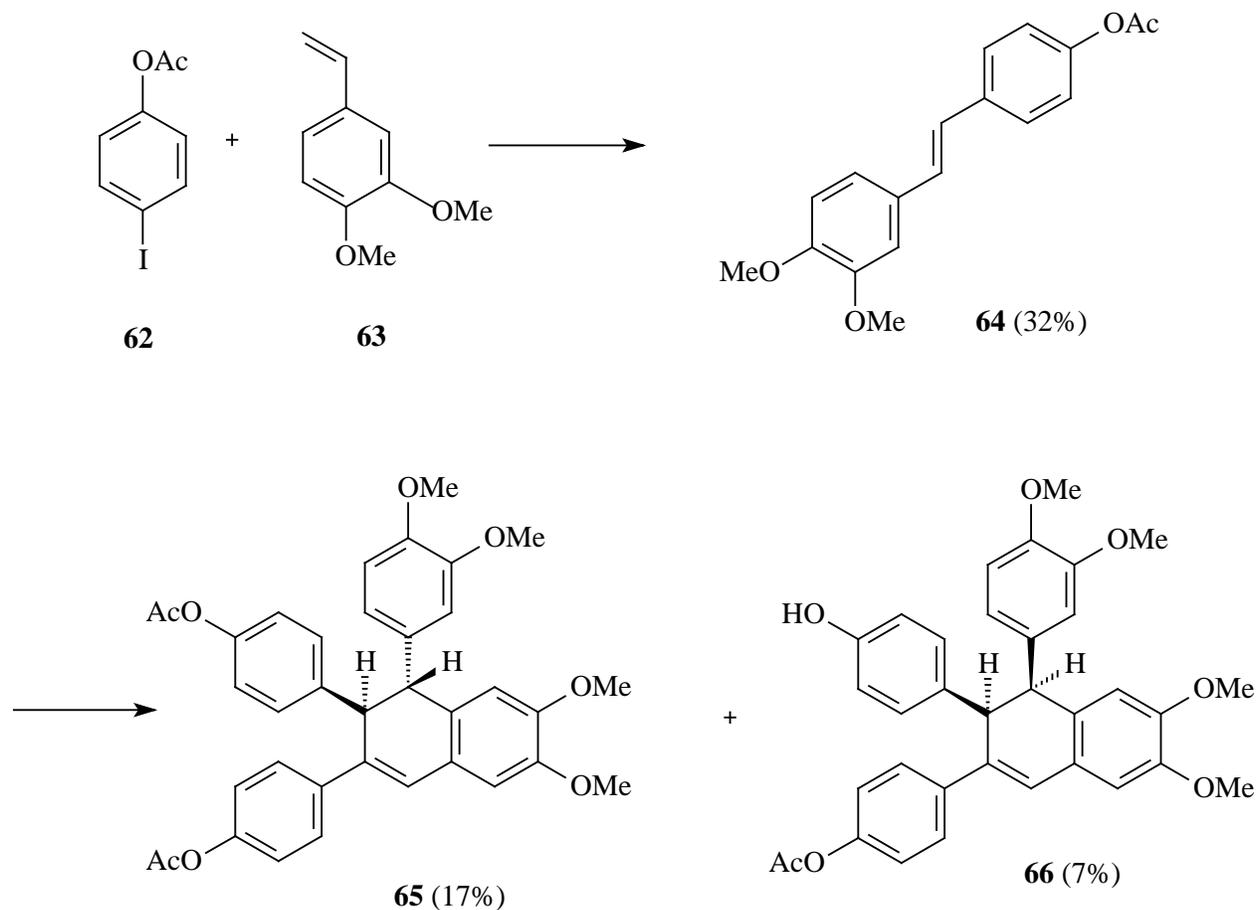
Resveratrol (**47**) is one of the unsymmetrical and (*E*)-geometrical stilbenes. Among the various methods used to synthesize unsymmetrical stilbenes, the Wittig reaction¹⁰⁸ is the most general methodology (**Scheme 4.2**). Wittig condensation of the appropriate aldehyde (for example **58**) with a phosphonium salt generated from *O*-protected 3,5-dihydroxybenzyl bromide (**55**) affords stilbene (**61**), as a mixture of (*E*) and (*Z*)-geometrical isomers in the ratio of 2:1. This *E/Z* mixture is efficiently converted to (*E*)-geometrical isomer by heating with a catalytic amount of I₂ in refluxing heptane for 12 hours.

¹⁰⁷ S. Eddarir, Z. Abdelhadi and C. Rolando. *Tetrahedron. Lett.*, **2001**, 42, 9127.

¹⁰⁸ V. P. Rao, A. K. Jen, K.Y. Wong, and K. J. Drost. *Tetrahedron Lett.*, **1993**, 34, 1747.

4.5. Stilbene oligomer synthesis

A FeCl₃-promoted sequential pericyclic pathway leading to a highly oxygenated oligostilbenoid dimer (incorporating two asymmetric centres) has been reported¹⁰⁹ (**Scheme 4.3**).



Scheme 4.3. Synthesis of the stilbene dimers

The stilbene monomer (**64**) was obtained in 32% yield by heating a mixture of 4-iodoacetophenone (**62**) and 3,4-dimethoxystyrene (**63**) in the presence of palladium dichloride, triphenylphosphine, potassium acetate and silver nitrate in DMF for seven days. Treatment of (**64**) with ferric chloride in dichloromethane (room temperature) gave the unnatural stilbenoid dimers 6,7-di(4-acetoxyphenyl)-2,3-dimethoxy-8-(3,4-dimethoxyphenyl)-7,8-dihydronaphthalene (**65**) and 6-(4-acetoxyphenyl)-7-(4-hydroxyphenyl)-2,3-dimethoxy-8-(3,4-dimethoxyphenyl)-7,8-dihydronaphthalene (**66**).

¹⁰⁹ N. F. Thomas, K. C. Lee, T. Paraidathathu, J. F. F. Weber, K. Awang, D. Rondeau and P. Richomme. *Tetrahedron*, **2002**, 58, 7201.

5. Monomers from *Pericopsis elata*

5.1. Introduction

Phytochemical studies of *P. elata* resulted in the isolation of isoflavonoids, chalcones, monomeric and dimeric stilbenes. The durability of the heartwood of *P. elata* and its high resistance to pathogens may be attributed to the presence of the stilbenes. Stilbenes act as uncoupling agents that inhibit oxidative phosphorylation, the main source of energy in decay.

The acetone and methanol extracts of the pulverized heartwood of *P. elata* afforded a complex mixture of phenolic compounds which was resolvable only after extensive enrichment and fractionation procedures. Derivatization (acetylation and methylation) of the fractions to attain an acceptable level of purity led to substantial losses, hence prohibiting reliable quantification of the constituents. The variety of compounds isolated comprised: flavanones (naringenin and eriodictyol), isoflavones (genistein and biochanin A), dihydrochalcone [(*R*)- α -4,2',4'-tetraacetoxydihydrochalcone], α -methyldeoxybenzoins (angolensin), stilbene monomers (piceatannol, resveratrol and isorhapontigenin) and six novel stilbene dimers [(*rel*-2,3-*trans*-2-(3,4-dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-6-[2-(3,5-dimethoxyphenyl)-*E*-1-ethenyl]benzodioxane, *rel*-2,3-*trans*-2-(3,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-4-[2-(3,4-dimethoxyphenyl)-*E*-1-ethenyl]-6-methoxy-2,3-dihydrobenzofuran, *rel*-2,3-*trans*-2-(3,4-diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-4-[2-(3,4-diacetoxyphenyl)-*E*-1-ethenyl]-6-acetoxy-2,3-dihydrobenzofuran, *rel*-2,3-*trans*-2-(3,4-dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-4-[2-(3,5-dimethoxyphenyl)-*Z*-1-ethenyl]-6-methoxy-2,3-dihydrobenzofuran, *rel*-2,3-*trans*-4-Formyl-2-(3,4-dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-6-methoxy-2,3-dihydrobenzofuran and *rel*-2,3-*trans*-2-(3,5-diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-6-[2-(3,5-diacetoxyphenyl)-*E*-1-ethenyl]-4-acetoxy-2,3-dihydrobenzofuran]

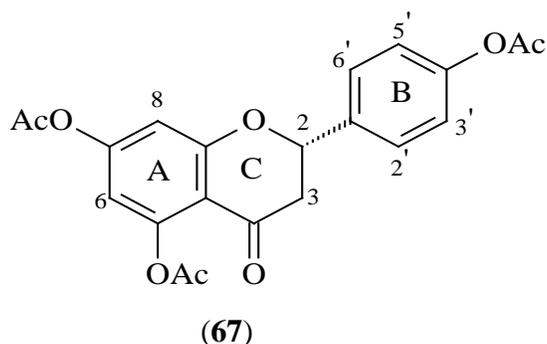
5.2 Flavanones

Acetylation and methylation followed by PLC separation of fractions A3 and B7 afforded *O*-acetyl and *O*-methyl derivatives of two known flavanones (**67** and **68**), respectively. Flavanones are characterized by the presence of the H-3 (two doublet of doublets, δ 3.00-3.15 and 2.69-2.85) and H-2 (doublet of doublets, δ 5.00-6.00) in their ^1H NMR spectra.¹¹⁴

The CD spectra (**Figures 5.1** and **5.2**) of compounds **67** and **68** were in line with the anticipated^{111,112} Cotton effect used to determine the absolute configuration at C-2. Thus the negative Cotton effect for the $\pi \rightarrow \pi^*$ transition at (~ 282 nm) and positive for the $n \rightarrow \pi^*$ transition at (~ 340 nm) are compatible with flavanones possessing a *2S* absolute configuration.¹¹³

5.2.1 5,7,4'-Triacetoxyflavanone

By far the most encountered natural flavanone is naringenin (5,7,4'-trihydroxyflavanone). This natural flavanone was isolated after acetylation and PLC separation of fraction A3 as the 5,7,4'-tri-*O*-acetyl derivative (**67**). This compound was identified by comparison of the ^1H NMR data of its *O*-acetyl derivative with data in literature.¹¹⁴



¹¹¹ D. G. Roux. *J. Biochem.*, **1963**, 87, 435.

¹¹² H. Arakawa and M. Nakazaki. *Ind. Chem.*, **1960**, 73.

¹¹³ W. Gaffield. *Tetrahedron*, **1970**, 26, 4039.

¹¹⁴ D. Ferreira, B. I. Kamara, E. V. Brandt and E. Joubert. *J. Agric. Food. Chem.*, **1998**, 46, 3406.

In the ^1H NMR spectrum (**Plate 1, Table 5.1**) of **67**, the aromatic AB spin system [δ 6.80 (d) and 6.56 (d)] and the aromatic AA'BB' spin system [δ 7.48 (d) and 7.18 (d)] correspond to the A- and B-rings, respectively. The heterocyclic C-ring protons show distinct spin systems at δ 5.51 (dd), H-2 (1H) and δ 3.06 (dd) and 2.79 (dd), H-3 (2H), characteristic of the flavanone nucleus.¹¹⁴

Ring	Proton(s)	(67) δ_{H} (ppm)
A	H-6	6.56 (d, J 2.5Hz)
	H-8	6.80 (d, J 2.5 Hz)
B	H-2',6'	7.48 (d, J 8.5 Hz)
	H-3',5'	7.18 (d, J 8.5 Hz)
C	H-2	5.51 (dd, J 14.0, 3.0 Hz)
	H-3 _(eq)	3.06 (dd, J 17.0, 14.0 Hz)
	H-3 _(ax)	2.79 (dd, J 17.0, 3.0 Hz)
	3 x -OAc	2.41 (s) 2.34 (s), 2.33 (s)

Table 5.1. ^1H NMR data of 5,7,4'-triacetoxyflavanone (**67**).

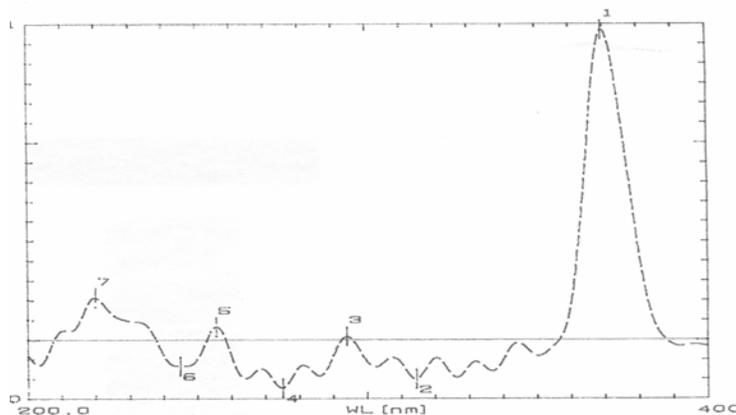
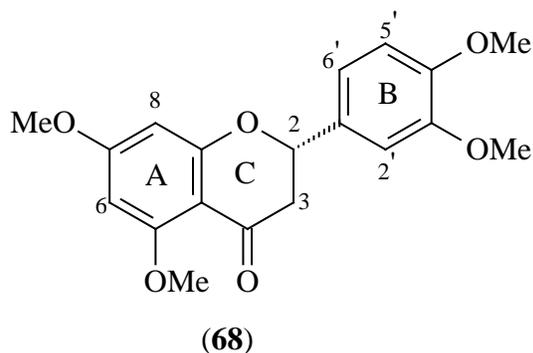


Figure 5.1: CD spectrum of compound (**67**).

5.2.2. 5,7,3',4'-Tetramethoxyflavanone

Eriodictyol the parent compound of several natural flavanones, was isolated after methylation and PLC separation of B7 as the *O*-methyl derivative (**68**).



In the ^1H NMR spectrum (**Plate 2, Table 5.2**) of **68**, the aromatic AB spin system [δ 6.17 (d) and 6.11 (d)] and the aromatic ABX spin system [δ 7.01 (dd), 7.00 (d) and 6.91 (d)] correspond to the A- and B-rings, respectively. The heterocyclic C-ring protons show distinct spin systems at δ 5.36 (dd), H-2 (1H) and δ 3.06 (dd) and 2.79 (dd), H-3 (2H), characteristic of the flavanone nucleus.¹¹⁴

Ring	Proton(s)	(68) δ_{H} (ppm)
A	H-6	6.11 (d, <i>J</i> 2.5Hz)
	H-8	6.17 (d, <i>J</i> 2.5 Hz)
B	H-2'	7.00 (d, <i>J</i> 2.5 Hz)
	H-5'	7.01 (dd, <i>J</i> 2.5, 8.5 Hz)
	H-6'	6.91 (d, <i>J</i> 8.5 Hz)
C	H-2	5.36 (dd, <i>J</i> 14.0, 3.0 Hz)
	H-3 _(eq)	3.07 (dd, <i>J</i> 17.0, 14.0 Hz)
	H-3 _(ax)	2.79 (dd, <i>J</i> 17.0, 3.0 Hz)
	4 x -OMe	3.94 (s) 3.92 (2 x s), 3.84 (s)

Table 5.2. ^1H NMR data of 5,7,3',4'-tetramethoxyflavanone (**68**).

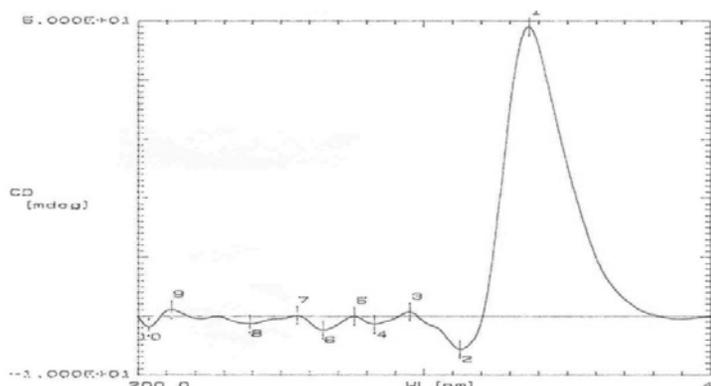


Figure 5.2: CD spectrum of compound (**68**).

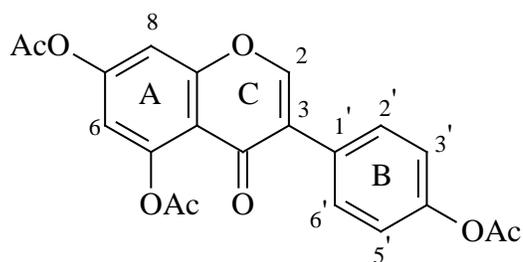
5.3. Isoflavones

Acetylation and PLC purification of fraction A3 afforded the peracetate derivatives of two known isoflavones (**69** and **70**). Their ^1H NMR spectra invariably display the singlet at δ 7.92 (**69**) and 7.89 (**70**) reminiscent of the vinylic H-2 resonance of isoflavones.

5.3.1. 5,7,4'-triacetoxyisoflavone

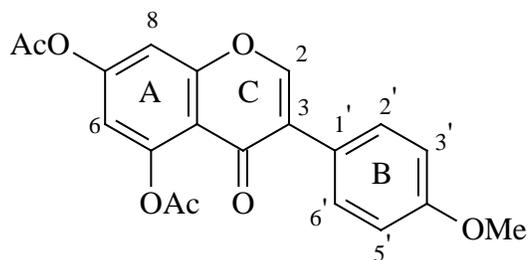
The most common isoflavone, genistein (5,7,4'-trihydroxyisoflavone), was isolated after acetylation and PLC separation as the tri-*O*-acetyl derivative (**69**). The ^1H NMR spectrum of **69** (Plate 3, Table 5.3) displays the 2-H singlet at δ 7.92, an AA'BB' spin system [δ 7.52 (d) and 7.18 (d)] attributed to the B-ring, the aromatic *meta*-coupled doublets [δ 7.85 (d) and 7.28 (d)] assigned to the A-ring and three accompanying acetoxy groups. Compound **69** was identified by comparison of the ^1H NMR data of its tri-*O*-acetyl derivative with data in the literature.¹¹⁵

¹¹⁵ J. L. Ingham., *Biochem. Syst. Ecol.* **1990**, 18, 329.



(69)

5.3.2. 5,7-diacetoxy-4'-methoxyisoflavone



(70)

The known biochanin A¹¹⁶ was isolated as a di-*O*-acetyl derivative (70) after acetylation and PLC separation of fraction A3 of the acetone extract. In the ¹H NMR spectrum of (70) (Plate 4, Table 5.3), the 4'-acetoxy of 5,7,4'-triacetoxy-isoflavone (69) is replaced by a natural methoxy group on the B-ring. An AA'BB' spin system [δ 7.42, (d) and δ 6.98, (d)] is attributed to the B-ring, the aromatic *meta*-coupled doublets (δ 7.26, d and 6.87, d) are assigned to the A-ring. Two acetoxy groups as well as one methoxy group are also displayed in the same spectrum. The n.O.e association of 4'-OMe with H-3',5' (Plate 4a) confirms the position of the methoxy group.

¹¹⁶ P. Pietta, P. Mauri, E. Manera and P. Ceva. *J. Chromatogr.*, **1990**, 513, 397

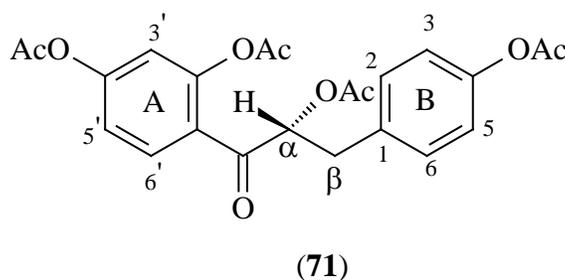
Ring	Proton(s)	(69) δ_H (ppm)	(70) δ_H (ppm)
A	H-8	7.28 (d, J 2.5 Hz)	7.26 (d, J 2.5 Hz)
	H-6	6.88(d, J 2.5 Hz)	6.87 (d, J 2.5 Hz)
B	H-2',6'	7.52 (d, J 8.5 Hz)	7.42 (d, J 8.5 Hz)
	H-3',5'	7.18 (d, J 8.5 Hz)	6.98 (d, J 8.5 Hz)
C	H-2	7.92 (s)	7.89 (s)
	OAc	2.44 (s), 2.37 (s), 2.34 (s)	2.44 (s), 2.37 (s)
	OMe		3.85 (s)

Table 5.3. ^1H NMR data of 5,7,4'-triacetoxyisoflavone (**69**) and 5,7-diacetoxy-4'-methoxyisoflavone (**70**).

5.4. Dihydrochalcones

5.3.1. (*R*)- α -4,2',4'-tetraacetoxydihydrochalcone

Dihydrochalcones bearing an oxygen substituent alpha to the carbonyl carbon are rare in nature. However, a known dihydrochalcone, (*R*)- α -4,2',4'-tetra-acetoxydihydrochalcone (**71**) was isolated after acetylation and PLC purification of fraction A2.



The ^1H NMR spectrum (**Plate 5, Table 5.4**) of **71**, displays a methylene group [$\text{H-}\beta_{(\text{eq})}$ (3.22 (dd),) and $\text{H-}\beta_{(\text{ax})}$ 3.14 (dd)] and $\text{H-}\alpha$ 5.99 (dd), which are characteristic resonances of the α -hydroxydihydrochalcone. The AA'BB' spin system at δ 7.28 (d) and 7.06 (d) is attributed to the B-ring and the ABX spin system [δ 7.77 (d), δ 6.71 (dd) and δ 6.81 (d)]

is assigned to the A-ring. The three aromatic acetoxy groups appear as singlets at δ 2.32 (s), 2.34 (2 x s) and the aliphatic acetoxy group resonates at δ 2.15 (s).

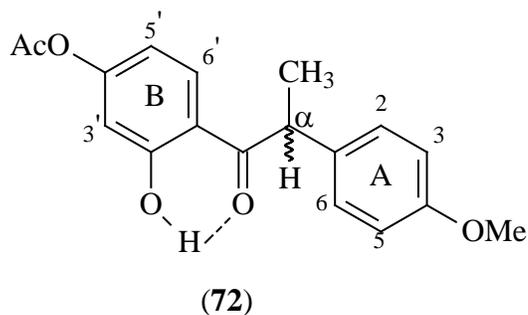
Ring	Proton(s)	(71) δ_H (ppm)
A	H-3'	6.81 (d, J 2.5 Hz)
	H-5'	6.71 (dd, J 8.5, 2.5 Hz)
	H-6'	7.77 (d, J 8.5 Hz)
	H- $\beta_{(eq)}$	3.22 (dd, J 15.0, 8.5 Hz)
	H- $\beta_{(ax)}$	3.14 (dd, J 15.0, 8.5 Hz)
	H- α	5.99 (dd, J 8.5, 2.5 Hz)
B	H-2,6	7.28 (d, J 8.5 Hz)
	H-3,5	7.06 (d, J 8.5 Hz)
	3 x OAc	2.34 (2 x s), 2.32 (s)
	OAc	2.15 (s)

Table 5.4. 1H NMR data of (*R*)- α -4,2',4'-tetraacetoxydihydrochalcone (**71**).

5.5. α -Methyldeoxybenzoin

Angolensin¹¹⁷ is an example of the α -methyldeoxybenzoin class of compounds. Co-occurrence with various isoflavonoids suggests that the α -methyldeoxybenzoin could be reduced forms of the isoflavonoid skeleton. A single α -methyldeoxybenzoin **72** was isolated after acetylation and PLC separation of fraction A3.

5.5.1. 4'-Acetoxy-2'-hydroxy-4-methoxy- α -methyldeoxybenzoin



¹¹⁷ F. E. King, T. J. King, and A. J. Warwich, *J. Chem. Soc.*, **1952**, 96, 1920.

The ^1H NMR spectrum (**Plate 6, Table 5.4**) of compound **72** displays an ABX spin system [δ 6.71 (d) 6.59 (dd) 7.82 (d)] allocated to the A-ring, an AA'BB' spin system [6.87 (d) 7.22 (d)] assigned to the B-ring, an aromatic acetoxy group at δ 2.30 (s) and a methoxy group at δ 3.79 (s). The conspicuous quartet at δ 4.63 and the doublet at δ 1.53 characteristic of the α -methyldeoxybenzoin compounds are displayed in the same spectrum. The n.O.e association of 4-OMe with H-3,5 (**Plate 6a**) confirms the position of the methoxy group. Due to the expected hydrogen bonding in structure (**72**), only one acetoxy group is observable after acetylation in the ^1H NMR spectrum. Compound (**72**) is confirmed to be angolensin by comparison with ^1H NMR data in the literature.¹¹⁸

Ring	Proton(s)	(72) δ_{H} (ppm)
A	H-3,5	6.87 (d, J 8.5 Hz)
	H-2,6	7.22 (d, J 8.5 Hz)
	H- α	4.63 (q, J 7.0 Hz)
	CH_3 - α	1.53 (d, J 7.0 Hz)
B	H-3'	6.71 (d, J 2.5 Hz)
	H-5'	6.59 (dd, J 8.5, 2.5 Hz)
	H-6'	7.82 (d, J 8.5 Hz)
	OMe	3.79 (s)
	OAc	2.30 (s)

Table 5.4: ^1H NMR data of 4'-acetoxy-2'-hydroxy-4-methoxy- α -methyldeoxybenzoin (**72**).

¹¹⁸ B. C. B. Bezuidenhout, E. V. Brandt and D. G. Roux. *J. C. S. Perkin 1*, **1980**, 2179.

5.6. Stilbenes

Two stilbene monomers that are considered to play a role as phytoalexins¹¹⁹, namely resveratrol and piceatannol were isolated from the heartwood of *P. elata*. Piceatannol the major phenolic constituent of *P. elata* (~16%) has been identified as the active ingredient of the species used in traditional herbal medicine¹²⁰ and as an antileukemic compound in the seeds of *E. lagascae*, which is used in folk medicine to treat cancer, tumors and warts.¹²¹ Resveratrol's biological properties include antifungal, antibacterial, anticancer, antiviral, oestrogenic, platelet aggregating and heart protecting activities.¹²² Isorhapontigenin isolated from *R. undulatum*¹²³ exhibited strong anti-allergic activity. Generally stilbenes act as uncoupling agents that inhibit oxidative phosphorylation, the main source of energy in decay.

5.6.1. Monomeric stilbenes

Stilbenes are phenolic compounds composed of two benzene rings linked by vinylic group. Irrespective of analyzing the samples in different solvents, overlapping of the aromatic protons in the ¹H NMR spectra of the stilbenes rendered structural elucidation difficult.

5.6.1.1. *trans*-4,3',5'-Triacetoxystilbene

The *trans*-4,3',5'-trihydroxystilbene, resveratrol (**47**), and its *cis* isomer are familiar plant metabolites isolated from red wine.^{124,125,126} The *trans*-tri-*O*-acetyl resveratrol derivative (**73**) was isolated after acetylation and PLC separation of fraction A3.

¹¹⁹ F. Wolter, A. Clausnitzer, B. Akoglu and J. Stein. *J. Nutr.*, **2002**, 132, 298.

¹²⁰ T. Tsururuga, T. T. Chun, Y. Ebizuka and U. Sankawa. *Chem. Pharm. Bull.* **1991**, 39, 3276.

¹²¹ N. R. Ferrigini, J. L. McLaughlin, R. G. Powell and C. R. Jr Smith. *J. Nat. Prod.*, **1984**, 47, 347.

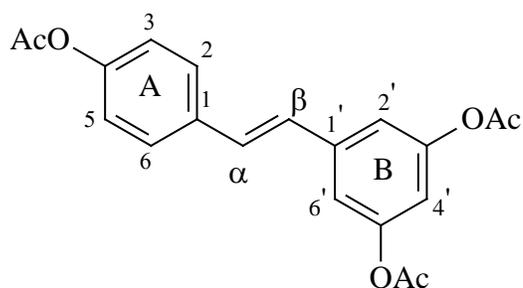
¹²² H. J. Lee, J. W. Seo, B. H. Lee, K-H. Chung and D. Y. Chi. *Bioorg. Med. Chem. Lett.*, **2004**, 14, 463.

¹²³ H. Matsuda, S. Tewtrakul, T. Morikawa and M. Yoshikawa. *Bioorg. Med. Chem.*, **2004**, 12, 4871.

¹²⁴ F. Mattivi and Z. *Lebensm. Unters. Forsch.*, **1993**, 196, 522.

¹²⁵ "Dictionary of Natural Products", ed. Buckingham, Chapman and Hall; London, **1994**, 2, 1783.

¹²⁶ G. J. Soleas, J. Dam, M. Carey and D.M. Goldberg., *J. Agric. Food. Chem.*, **1997**, 45, 3871.



(73)

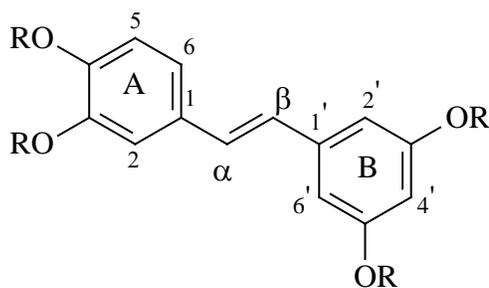
The ^1H NMR spectrum (**Plate 7, Table 5.6**) of **73** exhibits simple aromatic spin systems. An AA'BB' spin system [δ 7.51 (d) and 7.11 (d)] is attributed to the A-ring and an A₂B spin system [(δ 7.14 (d, 2H) 6.84(t)] is assigned to the B-ring. The doublets with a large coupling constant ($J = 16.0$) (H- α at δ 7.09 and H- β at δ 6.98) in the spectrum represent the *trans*-olefinic system. The three acetoxy groups resonate as a singlet (δ 2.33).

Ring	Proton(s)	(73) δ_{H} (ppm)
A	H-2,6	7.51 (d, J 8.5 Hz)
	H-3,5	7.11 (d, J 8.5 Hz)
	H- α	7.09 (d, J 16.0 Hz)
	H- β	6.98 (d, J 16.0 Hz)
B	H-2',6'	7.14 (d, J 2.5 Hz)
	H-4'	6.84 (t, J 2.5 Hz)
	3 x OAc	2.33 (3 x s)

Table 5.6. ^1H NMR data of *trans*-4,3',5'- triacetoxystilbene (**73**).

5.6.1.2. *trans*-3,4,3',5'- Tetraacetoxystilbene and *trans*-3,4,3',5'- tetrahydroxystilbene

Piceatannol¹²⁷ (*trans*-3,4,3',5'- tetrahydroxystilbene) also known as 3-hydroxyresveratrol or astringinine) was isolated as a free phenol (**48**) (major constituent of fraction B5 of the acetone extract) and as peracetate derivative (**74**) after acetylation and PLC separation of the same fraction.



(**48**) R = H

(**74**) R = Ac

The ¹H NMR spectrum (**Plate 8, Table 5.7**) (**48**) displays an ABX spin system at δ 7.09 (d) 6.82 (d) and 6.91 (dd) attributed to the A-ring and an A₂B spin system [δ 6.54 (d), 6.27 (t)] assigned to the B-ring. The large β coupling constant ($J = 16.0$) observed between H- α [δ 6.97 (d)] and H- β [δ 6.83 (d)] in the spectrum corresponds to the *trans*-olefinic system. The four hydroxy groups δ 8.05, 8.15 and 8.35 (x2) resonate as broadened singlets.

The ¹H NMR spectrum (**Plate 9, Table 5.7**) of the tetra-*O*-acetyl derivative (**74**), the 3-acetoxy analogue of **73** displays an ABX spin system δ 7.31 (d) 7.18 (d) and 7.33 (dd) attributed to the A-ring and A₂B spin system [δ 7.11 (d, 2H), 6.84 (t)] assigned to the B-ring. The large coupling constant ($J = 16.0$) observed between H- α at δ 7.02 (d) and H- β at δ 6.94 (d) in the spectrum corresponds to the *trans*-olefinic system. Four acetate groups are also displayed in the same spectrum.

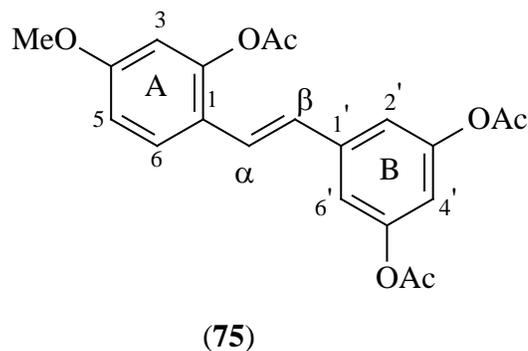
¹²⁷ J. Cunningham, E. Haslam and R. D. Haworth, *J. Chem. Soc.*, **1963**, 2875.

Ring	Proton (s)	(48) δ_H (ppm)	(74) δ_H (ppm)
A	H-2	7.09 (d, J 2.5 Hz)	7.31 (d, J 2.5 Hz)
	H-5	6.82 (d, J 8.5 Hz)	7.18 (d, J 8.5 Hz)
	H-6	6.91 (dd, J 8.5, 2.5 Hz)	7.33 (dd, J 8.5, 2.5 Hz)
	H- α	6.97 (d, J 16.0 Hz)	7.02 (d, J 16.0 Hz)
	H- β	6.83 (d, J 16.0 Hz)	6.94 (d, J 16.0 Hz)
B	H-2',6'	6.54 (d, J 2.5 Hz)	7.11 (d, J 2.5 Hz)
	H-4'	6.27 (t, J 2.5 Hz)	6.84 (t, J 2.5 Hz)
	4 x OH	8.35 (2 x bs), 8.15 (bs), 8.05 (bs)	
	4 x OAc		2.32 (s), 2.30 (2 x s), 2.29 (s)

Table 5.7. ^1H NMR data of *trans*-3,4,3',5'- tetrahydroxystilbene (**48**) and *trans*-3,4,3',5'- tetraacetoxystilbene (**74**).

5.6.1.3. *trans*-2,3',5'-Triacetoxy-4-methoxystilbene

trans-4,3',5'-Trihydroxy-3-methoxystilbene (isorhapontigenin) (**49**) was isolated after acetylation and PLC separation as the *trans*-*O*-acetyl derivative (**75**) from fraction A4.



The ^1H NMR spectrum (**Plate 10, Table 5.8**) of **75** displays an ABX spin system [δ 7.05 (d), 7.03 (d) and 7.08 (dd)] attributed to the A-ring and A_2B spin system [δ 7.15 (d, 2H) and 6.84 (t)] assigned to the B-ring. The large coupling constant ($J = 16.0$) observed between H- α at δ 7.07 (d) and H- β at δ 6.98 (d) in the spectrum represents the *trans*-olefinic system. Three acetoxy groups resonate at δ 2.35 (s), 2.33 (2 x s) and one methoxy group at δ 3.90 (s). The A_2B spin system and the three acetoxy groups displayed in the ^1H NMR of compound **74** (**Plate 9**) are accompanied by an additional methoxy group in compound **75** and the ABX spin system replaces the AA'BB' spin system. Compound **75** is therefore the 3-methoxy analogue of 4,3',5',-tri-*O*-acetyl resveratrol. This is confirmed by n.O.e association of 3-OMe with H-3 and H-5 (**Plate 10a**) of the ABX system.

Ring	Proton(s)	(75) δ_{H} (ppm)
A	H-3	7.05 (d, J 2.5 Hz)
	H-5	7.08 (dd, J 8.5, 2.5 Hz)
	H-6	7.03 (d, J 8.5 Hz)
	H- α	7.07 (d, J 16.0 Hz)
	H- β	6.98 (d, J 16.0 Hz)
	B	H-2',6'
H-4'		6.84 (t, J 2.5 Hz)
OMe		3.90 (s)
3 x OAc		2.35 (s), 2.33 (2 x s)

Table 5.8: ^1H NMR data of 2,3',5'-*trans*-triacetoxy-4-methoxystilbene (**75**).

6.1. Dimeric Stilbenes

6.1.1. Introduction

Although dimers from stilbene building blocks occur infrequently in nature, six dimeric stilbenes (**76-81, Figures 6.1 and 6.2**) have been isolated from the methanol and acetone extracts of *P. elata*. The structures of the dimeric stilbenes originate from different combinations of the two monomeric stilbenes, piceatannol (3,4,3',5'-tetrahydroxystilbene) (the major constituent ~16% of the phenolic content of *P. elata*), and 3,4,3',4'-tetrahydroxystilbene.

The relative stereochemistry at the chiral centers and the olefinic bonds in the dimers contribute to the variety of the structures. Elucidation of the structures is based mainly on spectroscopic data and is confirmed unambiguously by the synthesis of selected structures.

Natural coupling of the dimers is envisaged to follow a biosynthetic route involving two monomers where the olefinic carbons of one monomer couple with the vicinal 3,4- or 3,5-dioxygenated ring of the second monomer resulting in ether linkages and C-C bonds, forming dimers with different structural backbones (**Figures 6.1 and 6.2**). The ¹H NMR analysis of semi-purified fractions prior to derivatization demonstrated the absence of methoxy and acetoxy groups in the natural dimeric stilbenes.

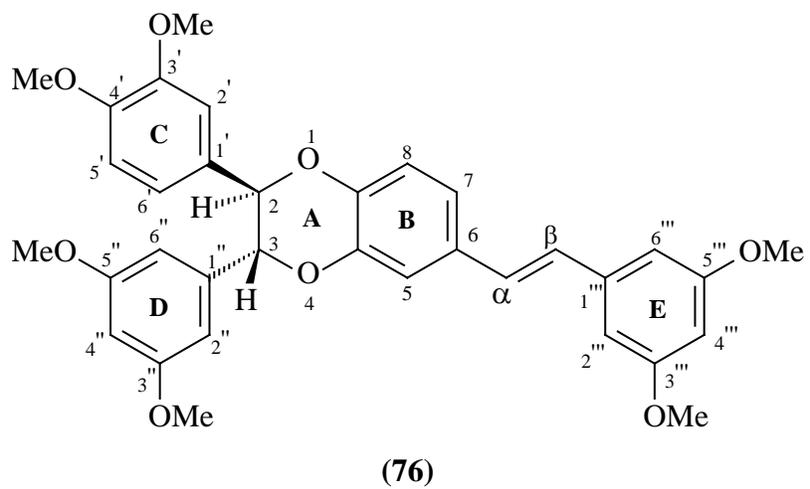
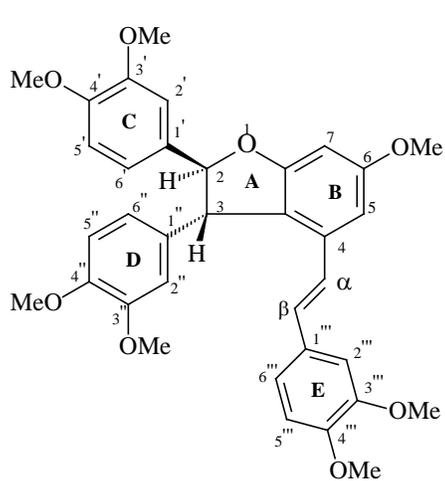
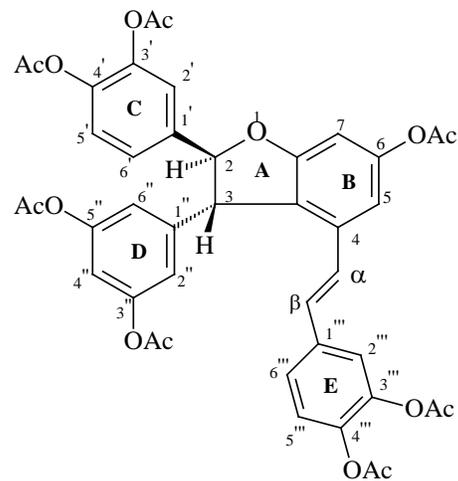


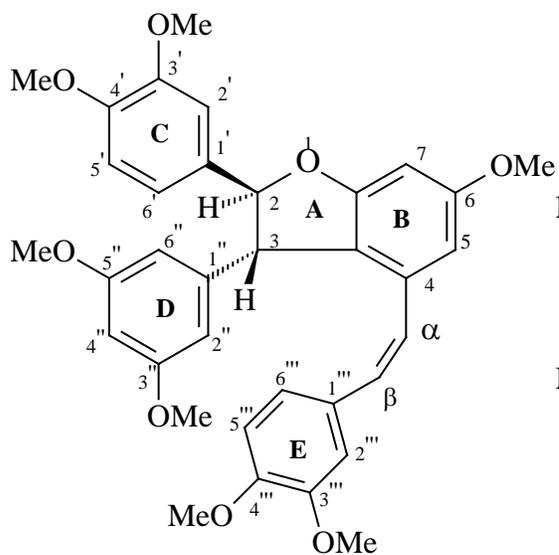
Figure 6.1: Dimeric stilbene with the dioxane coupling.



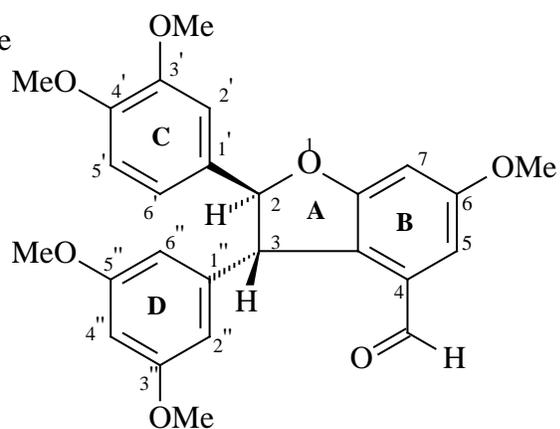
(77)



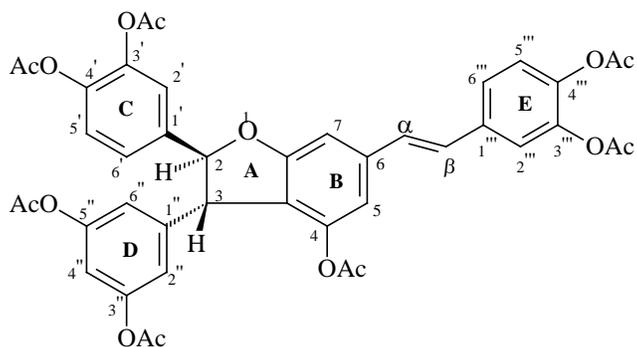
(78)



(79)



(80)



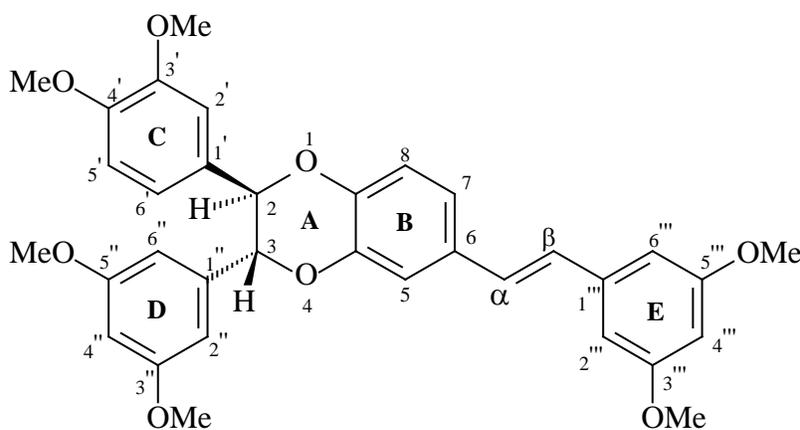
(81)

Figure 6.2: Dimeric stilbenes with the dihydrobenzofuran coupling.

6.2 Dimeric stilbenes with the dioxane coupling.

Following methylation of fraction 7 of the methanol extract a single dimeric stilbene with a dual ether linkage was isolated as a methoxy derivative **76**. The dimer possesses a dioxane fusion between the two units presumably originating from oxidative coupling of the oxygens of the catechol ring of one monomer with the vinyl bond of the second monomer, to give the benzodioxane A-ring. The ^1H NMR spectrum of derivative **76** (**Plate 11**) exhibits twelve aromatic protons, accompanied by six methoxy groups, a *trans*-disubstituted vinyl group and two deshielded aliphatic protons. The oxygenation patterns of the aromatic rings all belong to either a catechol- or resorcinol arrangement and the data suggest that it is a piceatannol (3,4,3',5'-tetrahydroxystilbene) dimer, which is probably its precursor.

6.2.1 *rel*-2,3-*trans*-2-(3,4-Dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-6-[2-(3,5-dimethoxyphenyl)-*E*-1-ethenyl]benzodioxane (**76**).



(**76**)

Methylation of fraction 7 of the methanol extract followed by PLC purification afforded the *o*-methyl derivative **76**. The ^1H and ^{13}C NMR spectral data of **76** (**Plates 11** and **11a**, **Table 6.1**), together with the ^1H - ^1H -COSY and HMQC spectra, permitted the assignment of two 3,5-dimethoxyphenyl rings (D and E) (A_2B spin systems), the 3,4-dimethoxyphenyl C-ring and catechol B-ring. The 3',4'-dimethoxy C-ring is designated by ^2J correlations of H-2' to C-3', H-5' to C-4', and ^3J of H-2 to C-2' and -6', while the 3'', 5''-dimethoxy phenyl D-ring is resolved by ^2J of H-2'',6'' to C-3'',5'' and ^3J of H-2'',6'' to C-3, (**Figure 6.4**, **Table 6.1**).

The ^1H NMR spectral data (**Plate 11**) also reveals the presence of two deshielded aliphatic methine protons [δ 4.88 (d, H-2) and 4.84 (d, H-3)]. The deshielding suggests that they could be attached to the benzylic carbons, each bearing an oxygen atom. This assumption is supported by the ^{13}C NMR chemical shifts [δ_{C} 81.3 ppm (C-2) and 80.7 ppm (C-3)] (**Table 6.1**). The coupling constant ($J = 8.0$ Hz) of the doublets corresponds to a *2,3-trans* relative configuration for the A-ring.¹²⁸ The molecular composition of **76** $\text{C}_{34}\text{H}_{34}\text{O}_8$ established from FABMS at m/z 570.2254 ($\text{M} + \text{H}$)⁺ is in accord with spectroscopic analyses made for compound **76**.

Significant n.O.e associations (**Figure 6.3, Plate 11b**) of H-2 with H-2'' and 6'' of the D-ring, as well as H-3 with H-2' and -6' of the C-ring confirm the proposed *2,3-trans* configuration. The same interactions confirm the assignments of the C- and D-rings.

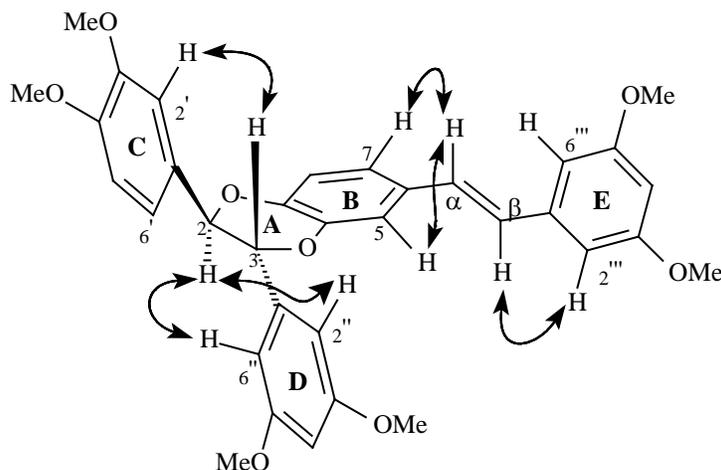


Figure 6.3: Relevant n.O.e associations of the methoxy derivative of the dimeric stilbene 76.

The *trans*-relationship of the olefinic protons H- α (7.03, d, $J = 14.0$ Hz) and H- β (6.93, d, $J = 14.0$ Hz) is indicated by the large coupling constant and n.O.e associations (**Figure 6.3**). Hence, strong n.O.e associations of H- α with H-7, and H- β with H-2''' (**Plate 11c**) confirms the B- and D rings hydroxylation patterns. 2D experiments [HMQC and HMBC (**Figure 6.4, Table 6.1**)], thus, allow the assignment of all proton and carbon signals (**Table 6.1**).

¹²⁸ G. R. Pettit, Y. Meng, C. A. Stevenson, D. L. Doubek, J. C. Knight, Z. Cichacz, R. K. Pettit, J-C.

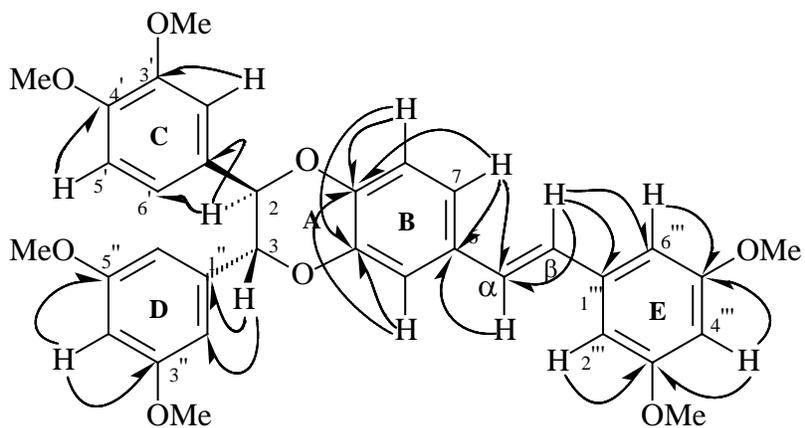


Figure 6.4: Relevant HMBC correlations of the methoxy derivative of the dimeric stilbene 76.

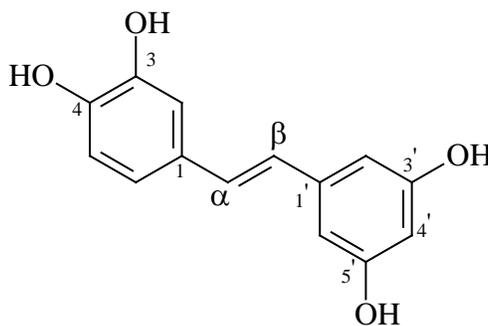
Ring	Protons	δ_H (ppm)	Carbons	δ_C (ppm)	2J (H→C)	3J (H→C)
A	2	4.88 (d, <i>J</i> 8.0)	2	81.3	C-1', C-3	C-2', C-6'
	3	4.84 (d, <i>J</i> 8.0)	3	80.7	C-2'', C-1''	C-2'', C-6''
	4a		4a	144.0		
	5	7.25 (d, <i>J</i> 2.5)	5	115.3	C-6, C-3a	C-8 α , C- α
B	6		6	131.6		
	7	7.12 (dd, <i>J</i> 8.5, 2.5)	7	120.8	C-6	C-8a, C- β
	8	7.05 (d, <i>J</i> 8.5)	8	117.8	C-8	C-3a
	8a		8a	144.3		
C	1'		1'	129.0		
	2'	6.62 (d, <i>J</i> 2.5)	2'	110.7	C-3'	
	3'		3'	149.1		
	4'		4'	149.6		
	5'	6.79 (d, <i>J</i> 8.5)	5'	111.1	C-4'	
	6'	6.71 (dd, <i>J</i> 8.5, 2.5)	6'	120.5	C-5'	C-2
D	1''		1''	138.7		
	2''	6.67 (d, <i>J</i> 2.5)	2''	104.8	C-3''	
	3''		3''	161.4		
	4''	6.40 (t, <i>J</i> 2.5)	4''	101.0	C-3'', C-5''	
	5''		5''	161.4		
	6''	6.67 (d, <i>J</i> 2.5)	6''	104.8	C-5''	
Olefinic	α	7.05 (d, <i>J</i> 16.0)	α	128.9	C-6, C- α	C-5, C-7
Olefinic	β	6.94 (d, <i>J</i> 16.0)	β	127.8	C-7', C-9'	C-2'', C-6''
E	1'''		1'''	139.9		
	2'''	6.25 (d, <i>J</i> 2.5)	2'''	106.0	C-3''', C-5'''	
	3'''		3'''	161.0		
	4'''	6.38 (t, <i>J</i> 2.5)	4'''	100.1	C-3''', C-5'''	
	5'''		5'''	161.0		
	6'''	6.25 (d, <i>J</i> 2.5)	6'''	106.0	C-3''', C-5'''	
	OCH ₃	3.88, 3.85 (x2), 3.78, 3.7 (x2)	OCH ₃	56.3 (x2) 55.8 (x4)		

Table 6.1. ¹H NMR (300 MHz), ¹³C HMR (75 MHz) and HMBC data of compound **76**.

6.3 Dimeric stilbenes with the dihydrobenzofuran coupling.

The dimeric stilbenes **77-81** all exhibit two prominent aliphatic doublets [δ 4.5-4.9 and δ 5.4-6.1] in their ^1H NMR spectra (**Plates 12, 13, 14, 15** and **16**) corresponding to signals at δ_{C} 55-58 and δ_{C} 91-94 ppm, respectively in the ^{13}C NMR spectra (**Plates 12a, 13a, 14a** and **16a**). The deshielded doublet at δ_{H} 5.4-6.1 (δ_{C} 91-94 ppm) suggests that the proton is attached to an oxygen bearing carbon in comparison to the methine proton [δ_{H} 4.5-4.9 (δ_{C} 55-58 ppm)] which is apparently not. Thus, the two methine protons in the ^1H NMR spectra belong to the dihydrofuran A-ring. Using this conclusion as the focal point, the structures of the dimeric stilbenes (**77-81**) were elucidated in conjunction with the 1D (^1H and ^{13}C NMR) and 2D experiments (HMQC, HMBC, NOESY and COSY).

Although all the dimeric stilbenes **77-81** display similar aliphatic doublets (H-2 and H-3) in their ^1H NMR spectra, spectroscopic data (HMBC, NOESY and HMQC) indicate that the coupling in the compounds **77-81** differs (**Figure 6.5**). In order to form the C-C bond between C-3 and C-3a (**Figure 6.5a and b**), coupling could occur between the olefinic carbon of one stilbene monomer with either C-4' or C-2'/'-6' of (**48**) in the second stilbene monomer. Compounds **77-80** have the same arrangement on the B-ring (**Figure 6.5a**) and their spectral data confirm that the C-C bond between C-3 and C-3a formed at C-2'/'-6' in the resorcinol ring, whereas for compound **81** the same C-C bond formed at C-4' in the resorcinol ring (**Figure 6.5b**).



(48)

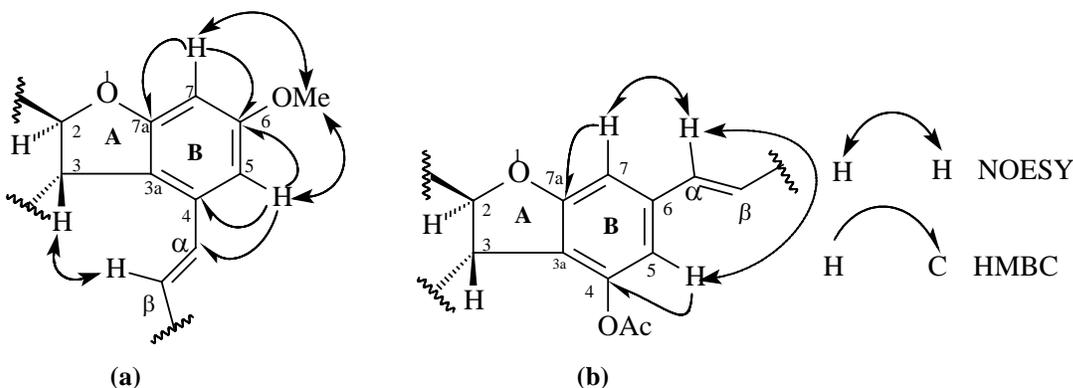


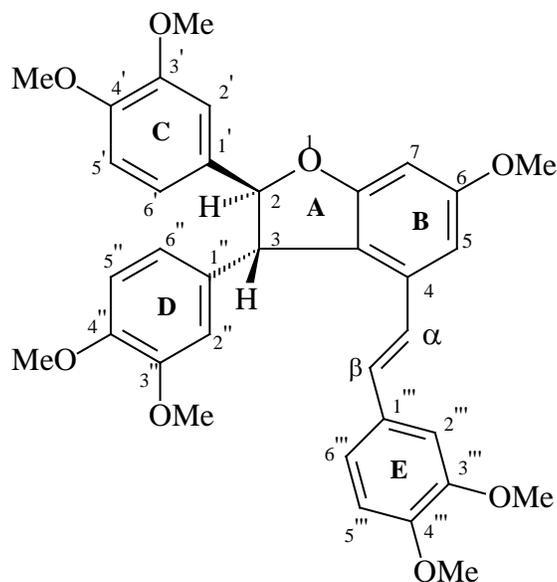
Figure 6.5. Relevant HMBC correlations and n.O.e associations confirming the products from the different couplings.

For compounds **77-80** there are strong HMBC correlations (**Figure 6.5a**) from H-7 to C-7a and C-6, and H-5 to C-4 and C- α , which confirm the hydroxylation pattern of the B-rings. However, for compound **81** the HMBC correlations show from H-7 to C-7a, and H-5 to the oxygen bearing carbon C-4. n.O.e Associations between H- α with both H-5 and H-7 (**Figure 6.5b**) confirm the hydroxylation pattern of the B-ring of **81**.

6.3.1 *rel-2,3-trans-2-(3,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-4-[2-(3,4-dimethoxyphenyl)-E-1-ethenyl]-6-methoxy-2,3-dihydrobenzofuran (77)*.

Methylation and PLC purification of fraction B7 afforded the *o*-methyl derivative of a novel dimeric stilbene **77**. Compound **77** has a molecular formula of C₃₅H₃₆O₈ revealed by FABMS at m/z 584.2410 (M + H)⁺. The ¹H NMR spectrum of **77** displays eleven aromatic protons, two olefinic protons, two deshielded aliphatic protons, and the seven methoxy groups. The aromatic protons are displayed as 3 sets of ABX spin systems for the C-, D- and E-rings, and an AB system for the B-ring (**Plate 12, Table 6.2**) all suggesting that 3,4,3',4'-tetrahydroxy stilbene and piceatannol (3,4,3',5'-tetrahydroxystilbenes) are its precursors. The prominent methine doublets at δ 5.52 and 4.45 ($J = 7.0$ Hz), characteristic of a dihydrobenzofuran A-ring¹²⁹ are allocated to H-2 and H-3, respectively. Allocation of all protons and carbons are *via* HMQC, HMBC, COSY and NOESY experiments.

¹²⁹ Y. M. Syah, N. S. Aminah, E. H. Hakim, N. Aimi, M. Kitajima, H. Takayama and S. A. Achmad. *Phytochemistry*, **2003**, *63*, 913.



(77)

HMBC correlations (**Figure 6.6, Table 6.2, Plate 12d**) of H-2 to C1', -2' and -6' and H-3 to C-2, -3a,-1'', -2'' and -5'' establish the C- and D-rings assignments. The E ring is allocated from the COSY and HMBC experiments, and confirmed by n.O.e association of H- β to H-2''' and -6''' (**Figure 6.7**).

Configuration at the two stereogenic centers (C-2 and C-3) is defined by prominent n.O.e interactions of H-2 with H-2'' and -6'' (**Figure 6.7, Plate 12c**), and H-3 with H-2', H-6' and H- β (**Plate 12b**) as a *trans* relative configuration. The same associations, together with n.O.e associations of H-2 with H-2' and H-6' and H-3 with H-2'' and H-6'' (**plates 12b and 12c**) further confirm the C and D-rings assignments.

FABMS revealed the base peak of compound **77** to be 584.2410 m/z ($M + H$)⁺, corresponding to the molecular formula of C₃₅H₃₆O₈.

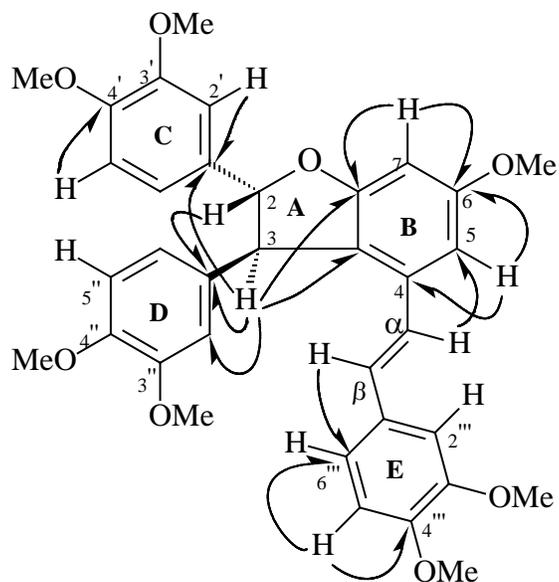


Figure 6.6: Relevant HMBC correlations of the *o*-methyl derivative of the dimeric stilbene 77.

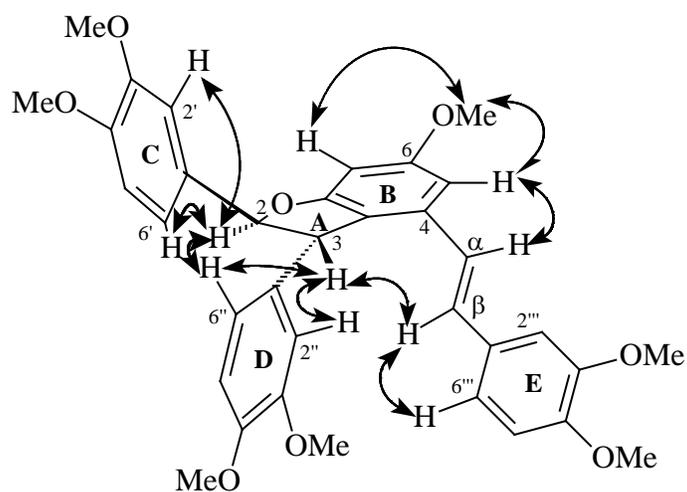
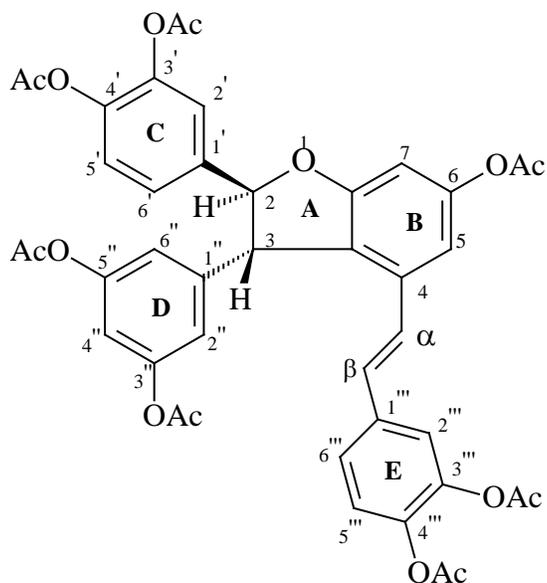


Figure 6.7: Relevant n.O.e associations of the *o*-methyl derivative of the dimeric stilbene 77.

Ring	Protons	δ_H (ppm)	Carbons	δ_C (ppm)	2J (H→C)	3J (H→C)
A	2	5.52 (d, J 7.0)	2	93.8	C-1'	C-2', C-6'
	3	4.54 (d, J 7.0)	3	57.7	C-1'', C-2, C-3a	C-1', C-7a, C-2'', 5''
B	3a		3a	120.2		
	4		4	136.0		
	5	6.76 (d, J 2.5)	5	120.9	C-4	C- α , C-3a C-6
	6		6	108.0		
	7	6.65 (d, J 2.5)	7	149.4	C-7a	C-6
	7a		7a	161.8		
C	1'		1'	146.0		
	2'	6.86 (d, J 2.5)	2'	118.6	C-1', C-3	C-2, C-6'
	3'		3'	149.8		
	4'		4'	149.8		
	5'	6.87 (d, J 8.5)	5'	111.5	C-4', C-6'	
	6'	6.87 (dd, J 8.5, 2.5)	6'	109.1	C-5'	C-1', 2'
D	1''		1''	134.0		
	2''	6.40 (d, J 2.5)	2''	106.5	C-3''	C-3, C-6''
	3''		3''	161.6		
	4''		4''	161.6		
	5''	6.39 (d, J 8.5)	5''	106.5	C-6''	
	6''	6.39 (dd, J 8.5, 2.5)	6''	99.2		C-3'', C-5''
Olefinic	α	6.89 (d, J 16.0)	α	129.7	C-5	C-4
Olefinic	β	6.68 (d, J 16.0)	β	123.7	C-1'''	C-6'''
E	1'''		1'''	130.6		
	2'''	6.50 (d, J 2.5)	2'''	95.6	C-1', C-3'''	C-6'''
	3'''		3'''	161.5		
	4'''		4'''	149.9		
	5'''	6.77 (d, J 8.5)	5'''	111.3	C-4'''	
	6'''	6.75 (dd, J 8.5, 2.5)	6'''	102.6	C-2'''	C-2'''
	OCH ₃	3.84 (x2), 3.87, 3.82, 3.79, 3.73 (x2)	OCH ₃	56.3 (X3), 56.0, 55.8, 55.7 (X2)		

Table 6.2. 1H NMR (300 MHz), ^{13}C NMR (75 MHz) and HMBC data of compound **77**.

6.3.2 *rel*-2,3-*trans*-2-(3,4-Diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-4-[2-(3,4-diacetoxyphenyl)]-*E*-1-ethenyl]-6-acetoxy-2,3-dihydrobenzofuran (78).



(78)

Following acetylation and PLC purification of fraction A7, compound **78** was isolated as a peracetate derivative. The ^1H NMR spectrum of **78** (Plate 13, Table 6.3) exhibits eleven aromatic protons, two *trans*-olefinic protons (δ 6.54 and 6.84 d, $J = 16.0$ Hz), two methine protons (δ 4.64 and 5.61, $J = 7.0$ Hz) and seven acetoxy groups. Four spin systems from eleven aromatic protons are assignable to the B-, C-, D- and E- rings. All the protons and carbons (Plate 13a) are allocated *via* HMQC, HMBC, NOESY and COSY experiments.

Assignment of the ABX system (Table 6.3) to the C-ring is confirmed by the strong n.O.e association (Figure 6.8, Plate 13b) of H-2 with H-6'. H-5' is established from its coupling (COSY) with H-6'. Two bonds removed for H-2' to the oxygen linked C-3' (144.4 ppm) and three bonds to C-4' (142.7 ppm) (HMBC) (Figure 6.9) confirm the ABX hydroxylation pattern of the C-ring.

The A_2B system of the D-ring is defined by a prominent triplet (resorcinol ring) at δ 6.66 (H-4'') and a doublet at δ 6.40 (H-2'',6'', 2H). The assignment is confirmed by both the

strong n.O.e associations (**Figure 6.8**) of H-3 with H-2'',6'' and H- β (**plate 13c**), as well as 2J of H-2'',6'' to C-3'',5'' and 3J of H-3 to C-2'',6'' (**Figure 6.9**).

n.O.e Association of H-2 with H-2'',6'' (**Figure 6.8**) establishes the relative 2,3-*trans* configuration. The B-ring protons H-5 and -7 are displayed as *meta*-doublets resonating at δ 7.01 and 6.95, respectively. Strong n.O.e association of H- α with H-5 (**Figure 6.8**), in conjunction with HMBC correlations of H- α with C-4 and C-3a confirm the allocation. Further indication is given by strong HMBC correlations of H-5 to C-6 and H-7 to the deshielded oxygen bearing carbons C-6 and C-7a (**plate 13d**). The remaining aromatic protons (**Table 6.3**) are assigned to the E-ring. Strong n.O.e association of H- β with H-2''' and -6''' confirm the allocation. The molecular formula of C₄₂H₃₆O₁₅ (M⁺ *m/z* 780.2054 (FABMS) is in agreement with the structure of compound **78**.

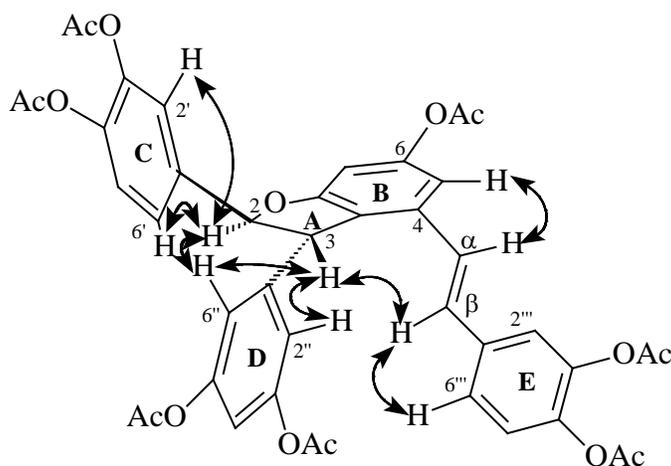


Figure 6.8: Relevant n.O.e associations of the peracetate derivative of the dimeric stilbene 78.

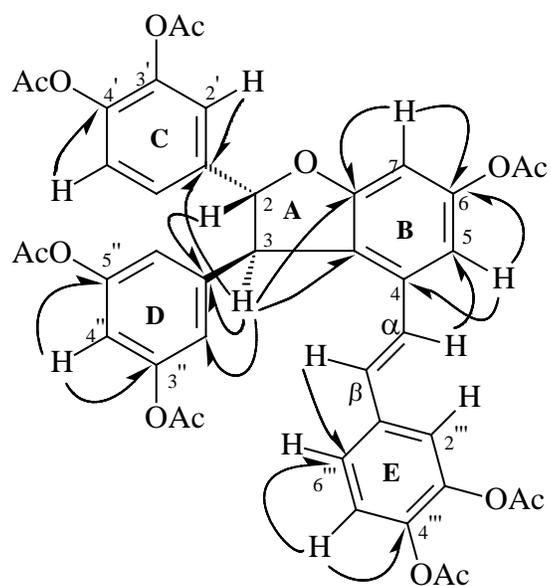


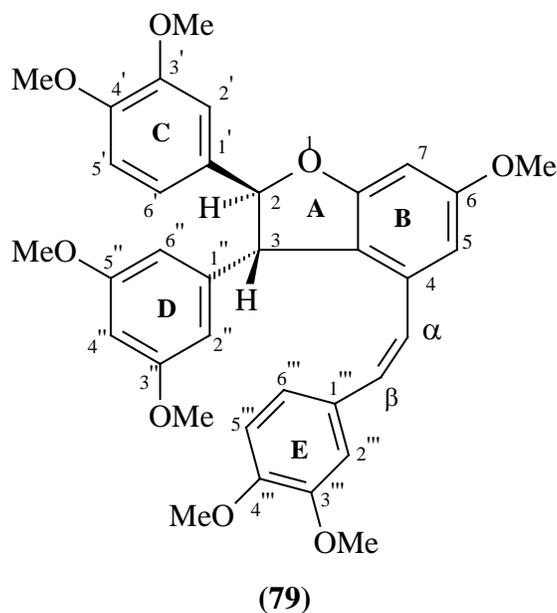
Figure 6.9: Relevant HMBC correlations of the peracetate derivative of the dimeric stilbene 78.

Ring	Protons	δ_H (ppm)	Carbons	δ_C (ppm)	2J (H→C)	3J (H→C)
A	2	6.03 (d, <i>J</i> 8.5)	2	88.2	C-1'	C-2', C-6'
	3	4.83 (d, <i>J</i> 8.5)	3	52.5	C-1'', C-2, C-3a	C-1', C-7a, C-2'', 5''
B	3a		3a	125.7		
	4		4	135.4		
	5	7.01 (d, <i>J</i> 2.5)	5	125.0	C-6	C- α
	6		6	135.3		
	7	6.95 (d, <i>J</i> 2.5)	7	121.8	C-7a	C-6
	7a		7a	161.2		
C	1'		1'	140.5		
	2'	6.72 (d, <i>J</i> 2.5)	2'	124.0	C-3'	C-1'
	3'		3'	142.6		
	4'		4'	142.1		
	5'	7.00 (d, <i>J</i> 8.5)	5'	123.3	C-4'	
	6'	6.96 (dd, <i>J</i> 8.5, 2.5)	6'	123.7	C-1'	
D	1''		1''	136.1		
	2''	6.40 (d, <i>J</i> 2.5)	2''	119.7	C-3'', 5''	
	3''		3''	151.4		
	4''	6.66 (t, <i>J</i> 2.5)	4''	114.5	C-3'', 5''	C-2'', 6''
	5''		5''	151.4		
	6''	6.40 (d, <i>J</i> 2.5)	6''	119.7		
Olefinic	α	6.88 (d, <i>J</i> 16.0)	α	130.5	C-4	C-3a
Olefinic	β	6.67 (d, <i>J</i> 16.0)	β	125.4	C-1'''	C- α
E	1'''		1'''	142.6		
	2'''	7.14 (d, <i>J</i> 2.5)	2'''	105.5	C-3'''	C-1'''
	3'''		3'''	153.1		
	4'''		4'''	141.9		
	5'''	7.10 (d, <i>J</i> 8.5)	5'''	122.3	C-4'''	
	6'''	6.99 (dd, <i>J</i> 8.5, 2.5)	6'''	112.0	β	C-4'''
	OCOCH ₃	2.37, 2.31, 2.30, 2.29, 2.28, 2.24, 2.23	OCOCH ₃	21.6, 21.5 (X2), 21.1 (X4)		
			O ₂ CCH ₃	169.4, 169.0 (x2), 168.9, 168.6, 168.4, 168.3		

Table 6.3. 1H NMR (300 MHz), ^{13}C NMR (75 MHz) and HMBC data of compound **78**.

6.3.3 *rel*-2,3-*trans*-2-(3,4-Dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-4-[2-(3,5-dimethoxyphenyl)]-*Z*-1-ethenyl]-6-methoxy-2,3-dihydrobenzofuran (79).

Compound **79** was isolated from the methanol extract B7 after methylation and PLC purification. The *o*-methyl derivative **79** displays the same number of aromatic and methine protons ^1H NMR (**Plate 14**) (**Table 6.4**) as compound **79**. The M^+ , m/z 584.2410 based on the FABMS corresponds to the molecular formula of $\text{C}_{35}\text{H}_{36}\text{O}_8$ that is expected for **79**. Allocation of the protons and carbons using the spectroscopic data (**Table 6.4**) indicates that compounds **78** and **79** have the same hydroxylation patterns for all the rings and only differ from each other with the geometric relationship of the olefinic protons. The olefinic protons in **79** (H- α , δ 6.10, J = 12.0 Hz and H- β , δ 6.25, J = 12.0 Hz) suggest that compound **79** is a *cis*¹²⁰ isomer of **78**. The smaller coupling constant (J = 12.0 Hz), and a strong n.O.e association of H- α with H- β (**Figure 6.10**, **Plate 14b**) confirm the *cis* isomer.



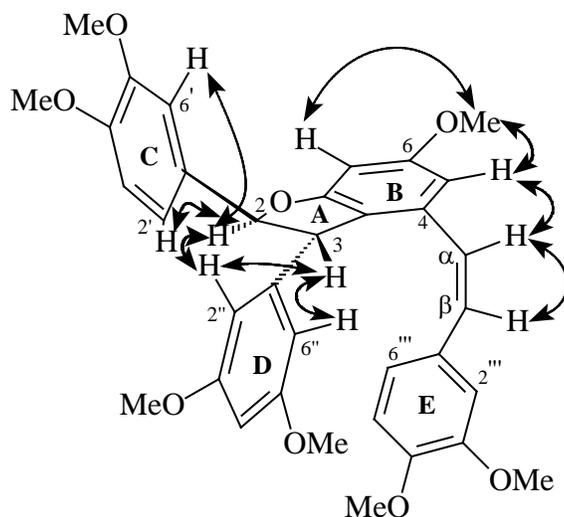


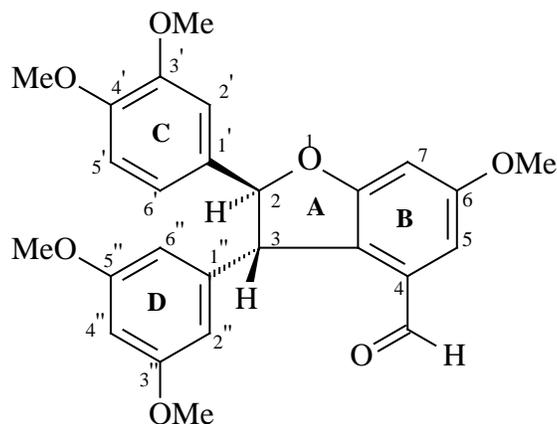
Figure 6.10: Relevant n.O.e associations of the *o*-methyl derivative of the dimeric stilbene **79.**

3J of H-2 to C-2' and -6' (HMBC) further confirms the assignments of the C-ring protons, while 3J of H-3 to C-2'' and -6'' confirms the D-ring assignments. The B-ring protons H-5 and -7 are established from the COSY and HMBC experiments. The ABX spin system assigned to the E-ring is established by the COSY experiments and confirmed by n.O.e interactions between H- β with H-6''' and H-2'', in conjunction with HMBC correlations of H- β to C-2''' and H-2''' to C-3'''. Thus, the novel compounds **78** and **79** are geometric isomers. The assignment of all the proton and carbon signals was established from the NOESY, COSY, HMQC, HMBC experiments.

Ring	Protons	δ_H (ppm)	Carbons	δ_C (ppm)	2J (H→C)	3J (H→C)
A	2	5.42 (d, J 7.0)	2	93.6	C-1'	C-2', C-6'
	3	4.03 (d, J 7.0)	3	57.5	C-1'', C-2, C-3a	C-1', C-7a, C-2'', 5''
B	3a		3a	120.2		
	4		4	131.5		
	5	6.41 (d, J 2.5)	5	107.5	C-6	C- α , C-3a
	6		6	161.0		
	7	6.46 (d, J 2.5)	7	97.7	C-6, C-7a	
	7a		7a	162.0		
C	1'		1'	145.5		
	2'	6.74 (d, J 2.5)	2'	120.9	C-1'	
	3'		3'	149.5		
	4'		4'	149.5		
	5'	6.81 (d, J 8.5)	5'	110.9	C-4'	
	6'	6.76 (dd, J 8.5, 2.5)	6'	109.1		
D	1''		1''	134.0		
	2''	6.20 (d, J 2.5)	2''	106.4	C-3'', 5''	C-1''
	3''		3''	161.0		
	4''	6.34 (t, J 2.5)	4''	149.5	C-3'', 5''	C-1''
	5''		5''	161.0		
	6''	6.20 (d, J 2.5)	6''	95.5	C-3'', 5''	C-1''
Olefinic	α	6.10 (d, J 12.0)	α	131.4	C-4	C-3a
Olefinic	β	6.25 (d, J 12.0)	β	126.4	C-1'''	C-6'''
E	1'''		1'''	136.5		
	2'''	6.64 (d, J 2.5)	2'''	113.0	C-3'''	
	3'''		3'''	148.5		
	4'''		4'''	149.5		
	5'''	6.67 (d, J 8.5)	5'''	111.5	C-4'''	C-3'''
	6'''	6.72 (dd, J 8.5, 2.5)	6'''	122.5		C-2''', C-4'''
	$O\overline{C}H_3$	3.89, 3.84, 3.83, 3.73 (x3), 3.61	$O\overline{C}H_3$	55.0-57.0 (x7)		

Table 6.4. 1H NMR (300 MHz), ^{13}C NMR (75 MHz) and HMBC data of compound **79**.

6.3.4 *rel*-2,3-*trans*-4-Formyl-2-(3,4-dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-6-methoxy-2,3-dihydrobenzofuran (80).



(80)

Methylation and PLC purification of fraction B7 of the methanol extract afforded the *O*-methyl ether derivative of compound **80**. Besides the two characteristic methine protons (δ 5.58, H-2 and δ 4.81, H-3)², which are prominent in the ¹H NMR spectrum (**Plate 15, Table 6.5**) of **80**, an exceptional peak at δ 9.77 allocated to the aldehyde is displayed. The aldehyde functionality replaces the vinylic group and the E-ring present in structures **77-79**. In the same ¹H NMR spectrum eight aromatic protons, two olefinic protons, and five accompanying methoxy groups are also displayed. The eight aromatic protons correspond to an ABX spin system (C-ring), an A₂B spin system (D-ring) and an AB spin system (B-ring) (**Table 6.5**). The small sample (0.9 mg) of compound **80**, obtained from the purifications did not allow analysis of the ¹³C NMR spectrum. Structure elucidation for **80** is based on the ¹H NMR, COSY and NOESY experiments as well as comparison of its ¹H NMR data with that of compounds **77-79**.

The ABX spin system assigned to the C-ring is confirmed by n.O.e associations (**Figure 6.11**) of H-2 and H-3 with H-2' and H-6'.

Ring	Proton	δ_H (ppm)
A	H-2	5.58 (d, J 6.0)
	H-3	4.81 (d, J 6.0)
B	H-5	6.98 (d, J 2.5)
	H-7	6.81 (d, J 2.5)
C	H-2'	6.86 (d, J 2.5)
	H-5'	6.84 (d, J 8.5)
	H-6'	6.84 (dd, J 8.5, 2.5)
D	H-2'',6''	6.29 (d, J 2.5)
	H-4''	6.37 (t, J 2.5)
	5 x OMe	3.90, s, 3.89, s, 3.68, s, 3.75 (x2), s
	<u>CHO</u>	9.77, s

Table 6.5. ^1H NMR (300 MHz) data of compound **80**.

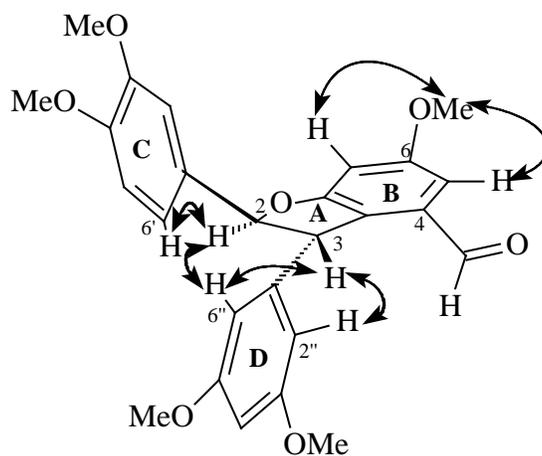


Figure 6.11: Relevant n.O.e associations of the *o*-methyl derivative of the dimeric stilbene **80**.

The relative *trans* configuration between H-2 and H-3 is established from the n.O.e association of H-3 with H-6', and H-2 with H-2'' and H-6'' (**Figure 6.11, Plate 15a**). H-5' of the ABX spin system is established from the COSY experiments. n.O.e Association of H-3 with H-6'' and H-2'' confirms the 3'',5''-dimethoxy D-ring. The *meta*-coupled protons at δ 6.98 (H-5) and 6.81 (H-7) are allocated to the B-ring. A strong n.O.e association of both H-5 and H-7 with 6-OMe (**Plate 15b**) confirms the allocations of H-5 and H-7. FABMS (M^+m/z 450.1679) corresponding to a molecular formula of $C_{26}H_{26}O_7$ corresponds with the structure of compound **80**.

6.3.5 *rel-2,3-trans-2-(3,5-Diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-6-[2-(3,5-diacetoxyphenyl)]-E-1-ethenyl]-4-acetoxy-2,3-dihydrobenzofuran (81)*

Compound **81** was obtained as a peracetate derivative following acetylation and PLC purification of fraction A7 of the methanol extract. In the 1H NMR spectrum (**Plate 16**), compound **81** displays the same number of aromatic protons as **77**, two aliphatic protons and seven acetoxy groups (**Table 6.6**). The thirteen protons in the aromatic region correspond to two ABX spin systems, an A_2B spin system, an AB spin system and two olefinic protons. ^{13}C and 1H NMR allocations (NOESY and HMBC experiments, **Figures 6.12 and 6.13**, respectively) (**Table 6.6**) confirmed that for compound **81** the substituted double bond is located on C-6 of the 2,3-dihydrobenzofuran ring. HMBC correlation (**Figure 6.13**) of only H-5 to the oxygen bearing carbon (C-4) and strong n.O.e association (**Figure 6.12**) of H-5 and H-7 to H- α (**plate 16b**) correlate with the hydroxylation pattern of the B-ring. Data from the NOESY, COSY, ^{13}C NMR, HMBC and HMQC experiments conclusively establish the hydroxylation patterns of the C-, D-, and E-rings (**Table 6.6**). FABMS revealed a molecular ion peak at m/z ($M + H$) $^+$ 780.2054 consistent with molecular formula of $C_{43}H_{40}O_{15}$ and confirms the structure of compound **81**.

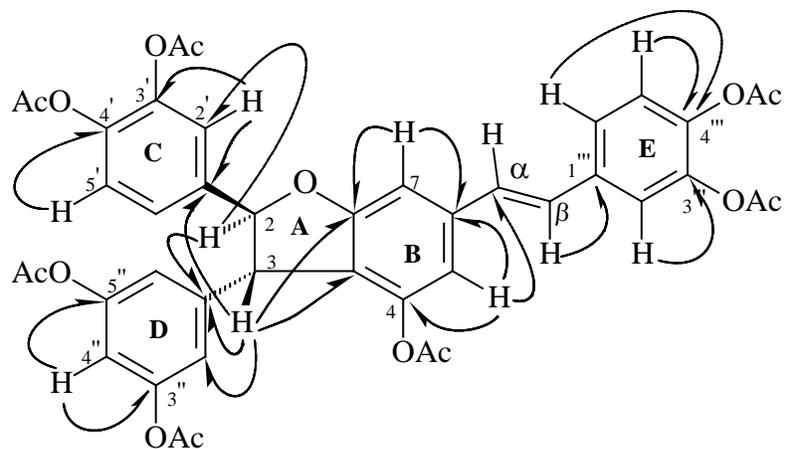


Figure 6.13: Relevant HMBC associations of the peracetate derivative of the dimeric stilbene 81.

Ring	Protons	δ_H (ppm)	Carbons	δ_C (ppm)	2J (H→C)	3J (H→C)
A	2	5.60 (d, J 7.0)	2	92.5	C-1'	C-2', C-6'
	3	4.61 (d, J 7.0)	3	57.0	C-1'', C-2, C-3a	C-1', C-7a, C-2'', 5''
B	3a		3a	125.3		
	4		4	142.1		
	5	7.08 (d, J 2.5)	5	121.9	C-4, C-6	C- α , C-7
	6		6	135.4		
	7	7.0 (d, J 2.5)	7	125.2	C-7a, C-6	C- α
	7a		7a	161.2		
C	1'		1'	144.6		
	2'	7.21 (d, J 2.5)	2'	124.2	C-1'	
	3'		3'	142.7		
	4'		4'	142.3		
	5'	7.20 (d, J 8.5)	5'	121.0	C-4'	
	6'	7.19 (dd, J 8.5, 2.5)	6'	124.0		C-2', C-5'
D	1''		1''	139.7		
	2''	6.88 (d, J 2.5)	2''	119.0	C-3'', C-5''	C-4''
	3''		3''	152.1		
	4''	6.92 (t, J 2.5)	4''	115.3	C-3'', C-5''	
	5''		5''	152.1		
	6''	6.88 (t, J 2.5)	6''	119.0		
Olefinic	α	6.54 (d, J 16.0)	α	130.2	C-6	C-7
Olefinic	β	6.85 (d, J 16.0)	β	125.2	C-1''	C-6'''
E	1'''		1'''	136.0		
	2'''	6.68 (d, J 2.5)	2'''	103.6	C-6'''	
	3'''		3'''	142.3		
	4'''		4'''	152.5		
	5'''	7.10 (d, J 8.5)	5'''	124.0	C-4'''	
	6'''	6.93 (dd, J 8.5, 2.5)	6'''	111.1	C-5'''	C-4'''
	OCOCH ₃	2.35, 2.30 (x2), 2.29, 2.27(x2)	OCOCH ₃	21.6, 21.5 (X2), 21.1 (X2), 21.0 (X2)		
			OCOCH ₃	169.9, 169.1 (x2), 168.7, 168.6, (x2), 168.5		

Table 6.6. 1H NMR (300 MHz), ^{13}C NMR (75 MHz) and HMBC data of compound **81**.

Summary

The bark of *Afromosia elata* has (family name) been used byfor the or as
Previous phytochemical studies (Ferreira et al) of *Afromosia elata* resulted in isolation of isoflavonoids, flavonoids, stilbene monomers and chalcones. Among the previously known isolates, stilbenes are known to have biological activities such as, act as antioxidants (Ref), antifungal (REF), and antimicrobial inhibitors (REF). They possess COX-1 and COX-2 inhibitory effects (REF), affect lipid peroxidation (REF), LDL oxidation (REF), and function as phytoalexins (REF) among other activities. Dimers with resveratrol as the building block have been isolated from different sources (REFERENCES)

The present paper reports the composition of the MeOH and acetone extracts of *A. elata*. Together with the known monomeric stilbenes (.....), isoflavonoids (.....), chalcones (.....) and a flavanone (.....), new compounds, namely a stilbene, (3,4,3',4'-tetra acetoxystilbene), a stilbenoid (.....), and eight dimeric stilbenes (.....) have been isolated.

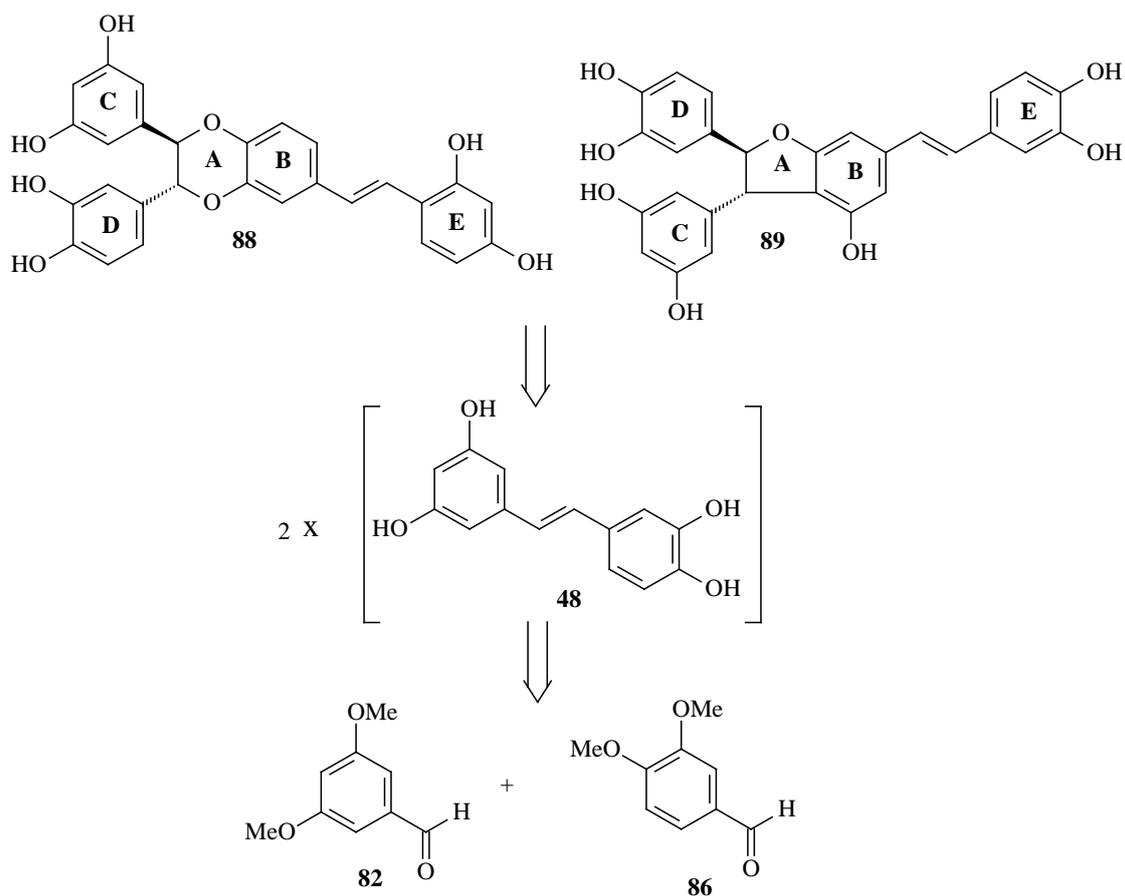
The aim of this work was to isolate, elucidate and establish the phytochemical composition of *Afromosia elata*, and relate the findings to the current uses of the plant and claimed physiological properties of the stilbenes, which are the major constituents.

7. SYNTHESIS

7.1 Introduction

Structural elucidation of the six dimeric stilbenes from *P. elata* proved to be difficult often due to overlapping of the aromatic protons in their ^1H NMR spectra (**Plates 11, 12, 13, 14, 15** and **16**). With the aim of improving the resolution of the overlapping aromatic protons, the samples were investigated in different deuterated solvents. This, however, did not necessarily solve the problem. Although the structural elucidation of the six novel stilbene dimers by spectroscopic methods was considered to be satisfactory, selected compounds were synthesized to confirm unequivocally the proposed structures.

The route considered for the synthesis of the dimeric stilbenes was conceived to proceed *via* oxidative coupling of the olefinic protons of one stilbene monomer with the catechol or the resorcinol ring of the second stilbene monomer according to the retrosynthetic pathway (**Figure 7.1**).



Scheme 7.1: Retrosynthetic pathway to stilbene dimers

The most common synthetic protocol for the synthesis of the monomeric stilbenes for example, piceatannol (**48**), proceeds *via* the Wittig reaction.¹⁰⁹ Thus, the stilbene monomer could be obtained following reduction and bromination of commercially available aldehyde. Synthesis of the dimeric stilbenes was accordingly implemented *via* the protocol in **Scheme 7.1**.

7.2. Synthesis of the stilbene monomer (**48**)

3,5-Dimethoxybenzyl alcohol (**83**) was obtained in 96% yield by the reduction of 3,5-dimethoxybenzaldehyde (**82**) using NaBH₄ in ethanol. Structure of the alcohol (**83**) was confirmed by ¹H NMR where the spectra showed the disappearance of a signal 9.81 ppm corresponding to aldehydic group and the appearance of a signal at 4.64 ppm corresponding to the methine protons indicating the formation of 3,5-dimethoxybenzyl alcohol (**83**).

During the synthesis of the phosphonium salts the alcohol (**83**) was treated with phosphorus tribromide in dry THF to yield 3,5-dimethoxybenzyl bromide (**84**) in 98% yield. 3,5-Dimethoxybenzyl bromide (**84**) was refluxed with excess triphenylphosphine in dry acetonitrile and 3,5-dimethoxybenzyl triphenylphosphonium bromide (**85**) was obtained in 40% yield. The structure of phosphonium bromide was (**85**) was confirmed by ^1H NMR, appearance of signals in 7.87 ppm region corresponding to 15 protons of triphenyl phosphine and the downfield shift of methine protons due to the electron-withdrawing phosphorus group.

3,5-Dimethoxybenzyl triphenylphosphonium bromide (**85**) was subjected to the Wittig reaction with appropriate aryl aldehyde (**86**) and the resulting monomeric stilbene (**87**) was obtained in 40% yield. Purification of the stilbene by pTLC or flash chromatography resulted in the decomposition of the stilbene and low yields. The phosphonium salt reacted with the appropriate aryl aldehyde (**86**) to yield the desired stilbene monomer, albeit in modest yield (**Scheme 7.1**), probably due to the electron-delocalizing phenyl substituent of the benzyl group which decreases the reactivity of the phosphoranes. A probable side reaction involving attack of the base directly on phosphorus may further be responsible for the low yields (**Figure 7.1**).

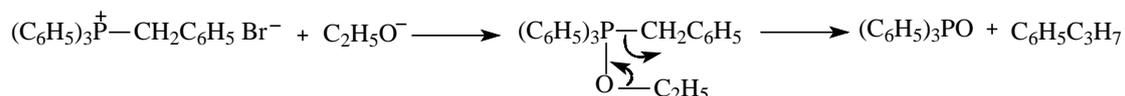
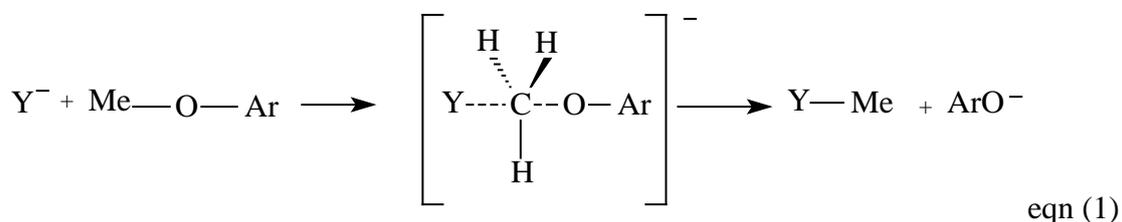


Figure 7.1. Direct attack on phosphorus by the base.

During the multi-step synthesis of the monomeric stilbene, the phenolic hydroxyl groups were protected as methyl ethers. Due to the stability of aryl methyl ethers difficulties were encountered during the regeneration of the parent phenol. The methods available for the regeneration of phenols from aryl methyl ethers include cleavage by acidic reagents, by organometallic compounds, by treatment with alkali metals, or by reaction with nucleophiles. Cleavage by acidic reagents required harsh reaction conditions and the methylated stilbene could be protonated. Demethylation by organometallic compounds and alkali metals requires the use of high temperatures and long reaction times, mostly under pressure, giving partial demethylation.

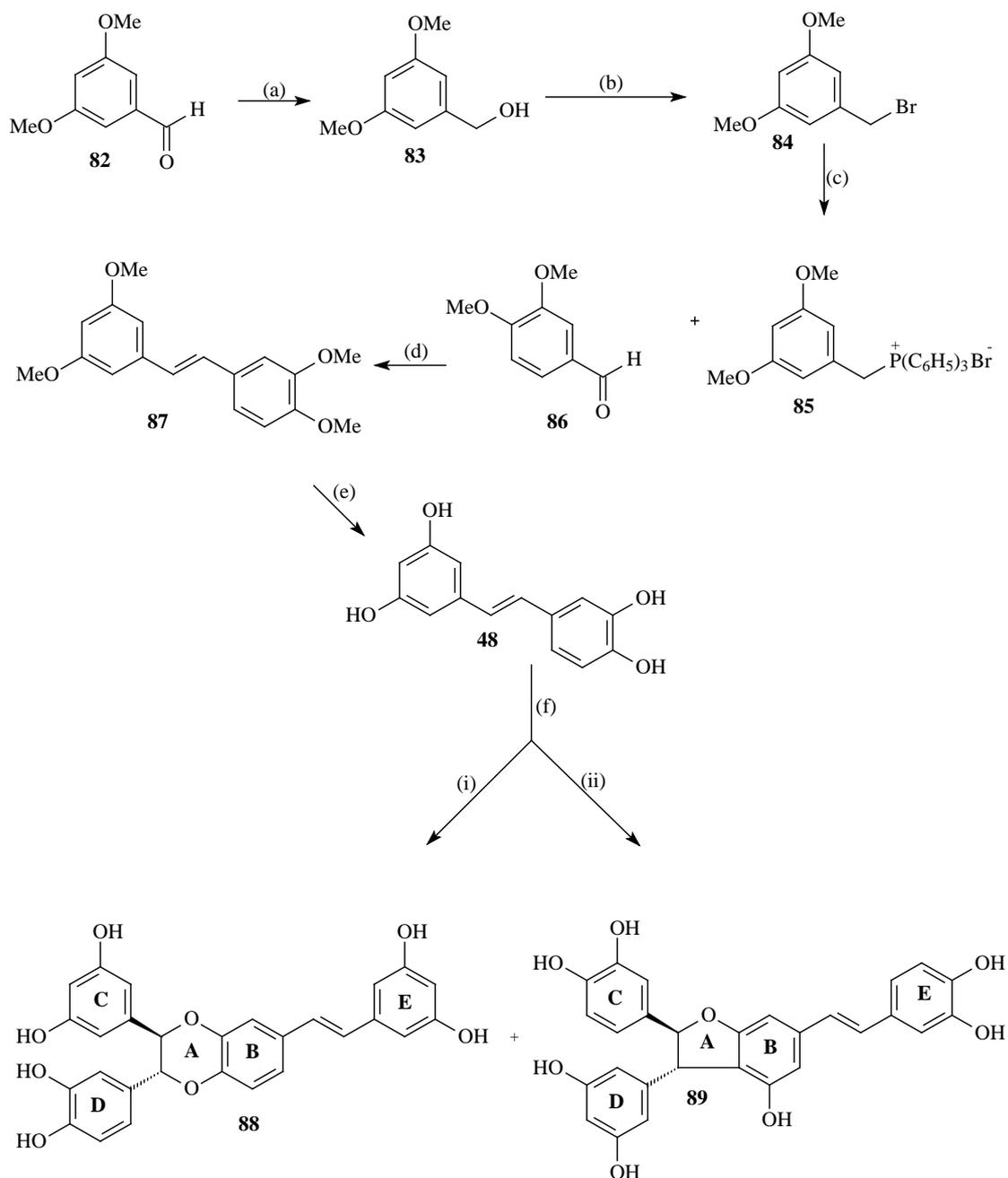
Cleavage by nucleophiles was chosen as the best method and sodium ethoxide was used as a suitable reagent because of the ease with which the phenol can be separated from excess reagent and the volatile methyl ethyl sulphide (Y-Me) (eqn. (1), Y = EtS).



Since demethylation of aryl methyl ethers by nucleophilic reagents proceeds by S_N2 displacement on the alkyl methyl group (eqn.(1)) dipolar aprotic DMF was chosen as a solvent to enhance the nucleophilicity of ethoxide anion, and it is stable to strong bases at reflux temperatures. DMF is also water soluble, thus, allowing easy recovery of phenolic compounds.

7.3. Synthesis of the stilbene dimers (88 and 89)

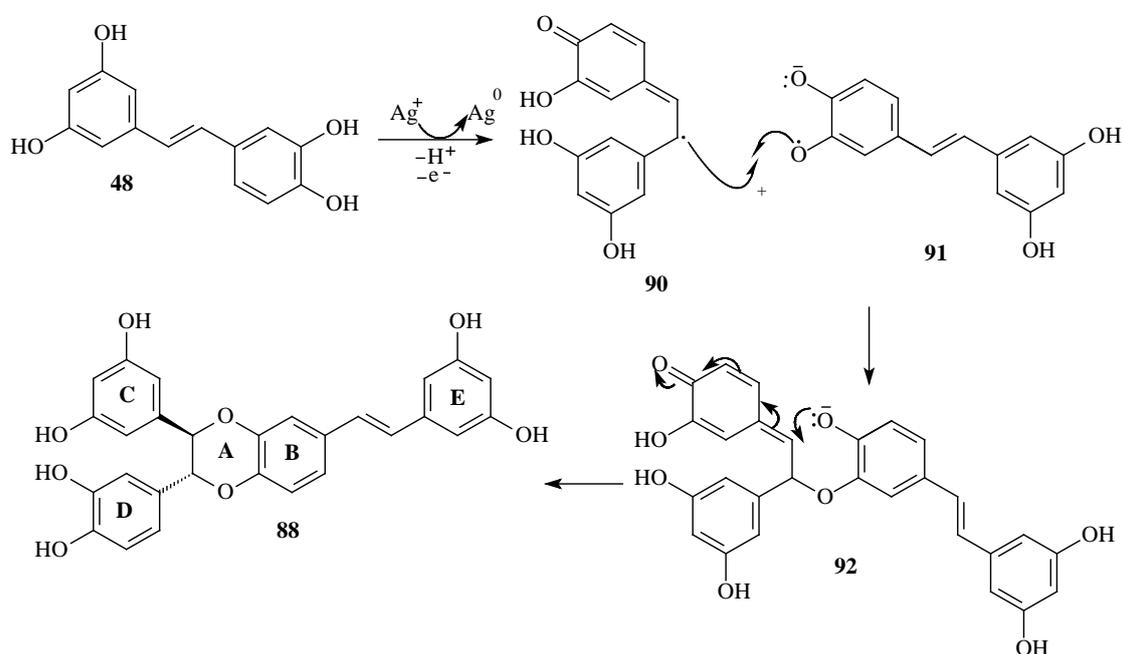
The free phenolic stilbene (**48**) was heated with AgOAc (60 °C, 30 min) in dry methanol. The use of methanol as a solvent was the most effective for the smooth formation of the dimers because of the good solubility of AgOAc in methanol, as to opposed other organic solvents, such as THF, acetone and acetonitrile. PLC separation of the mixture yielded the free phenols **88** and **89**. The reaction is envisaged to proceed *via* two routes (**48** to **88** and **48** to **89**) (Scheme 7.2) probably by a free radical mechanism (Scheme 7.3).



Scheme 7.2. Reagents and conditions: (a) NaBH₄, EtOH; (b) PBr₃, THF; (c) PPh₃, DMF; (d) NaOMe, DMF; (e) EtSNa, DMF; (f) AgOAc, MeOH

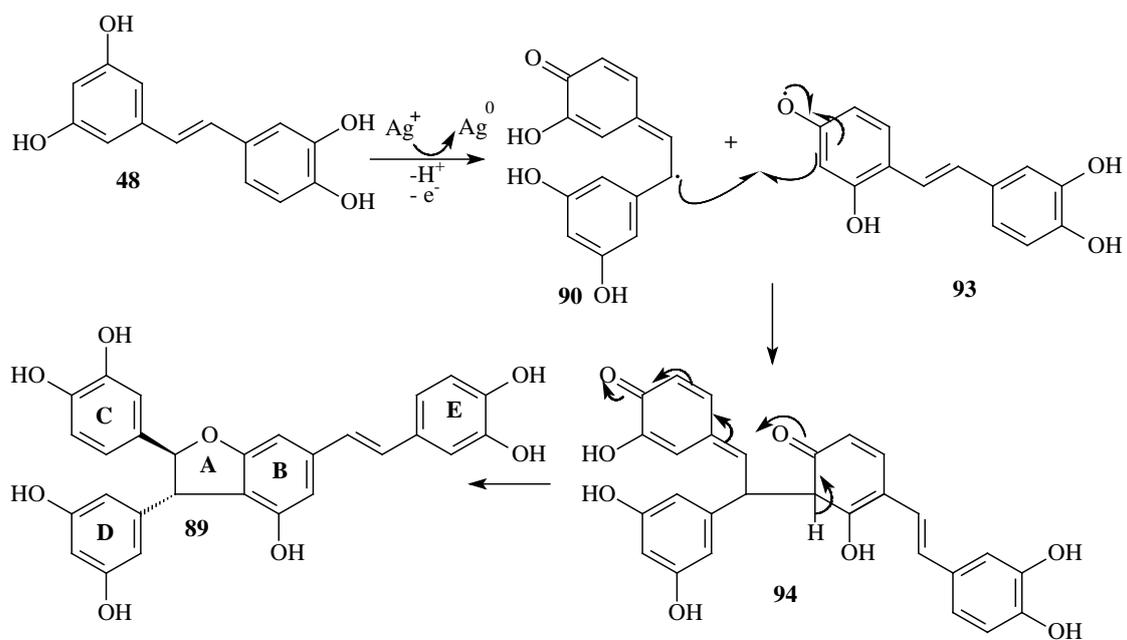
The anticipated course of reaction is attributed to the heat sensitive AgOAc (the oxidizing agent) which yields Ag⁺ and initiates the mechanism by removing an electron from the olefinic system **48** to form a benzylic radical (**90**). Deprotonation of 4-hydroxy group of the catechol ring of a second molecule of the stilbene forms the stabilized phenoxyl radical (**91**). During the formation of (**88**) the phenoxyl radical of the catechol ring (**91**) couples with the benzylic radical (**90**) to yield (**92**). The subsequent nucleophilic attack occur using the lone pair on oxygen to give **88**.

Methylation of **88** yielded **76**. ^1H NMR of the methylated derivative is identical to the *O*-methyl ether derivative of the isolated compound **76**.



Scheme 7.3. Proposed mechanism for the formation of **88**

Compound **89** forms when the resorcinol ring of the stilbene monomer (**48**) is involved in the coupling instead of the catechol ring (**Scheme 7.4**). The benzylic radical (**90**) attacks the position *ortho* to the phenoxy radical (**93**) generating a *para* quinone (**94**) which is highly susceptible to cyclization. Loss of a proton and a simultaneous ring closure yields compound **89** which was subsequently acetylated to (**81**). The ^1H NMR of the peracetate derivative of **89** was identical to that of the isolated compound **81**. The novel compounds (**76**) and (**81**) therefore, have been successfully synthesized and their structures unequivocally confirmed.



Scheme 7.4 Proposed mechanism for the formation of **89**

CHAPTER 8

8. Standard experimental techniques

Unless otherwise stated the following techniques were applied through the course of this study.

8.1. Chromatographic techniques

8.1.1. Paper chromatography

Two-dimensional paper chromatograms were conducted on Whatman no 1 paper (28.5X 46cm) in two directions, using water-saturated butan-2-ol in the first direction and 2% (v/v) acetic acid in the second direction. After development, the chromatograms were air dried and investigated by UV-light and spraying reagents.

8.1.2. Column chromatography

Separations on Sephadex LH-20 were carried out on various column sizes at differing flow rates as specified. The Sephadex LH-20 was prepared by soaking it in the eluant [ethanol or methanol/water (50/50)] for 24-hours. The crude extract in a minimum amount of the eluant was applied to the packed Sephadex LH-20 column. Column fractions were eluted at a flow rate of 0,5 ml/min, and fractions of 15 ml were collected with an ISCO (model 273) automatic fraction collector.

Merck Kieselgel 60 column chromatography separations were performed on various column sizes at different flow rates under gravity. The glass column was charged with this suspension of Kieselgel 60 and the crude extract, dissolved in a minimum of the appropriate solvents applied to the column. Columns were eluted at a flow rate of approximately 0.5 ml/min and fractions of 15 ml were collected with an ISCO (model 273) automatic fraction collector.

Flash column chromatography (FCC) was performed on a glass column (5 cm diameter) charged with 100g of Merck Kieselgel 60 (230-400 mesh) for every 1 g of the crude material. Air was displaced by elution with the appropriate solvent under N₂-pressure (*ca.* ~ 40kPa). The

crude product was dissolved in a minimum of the appropriate solvent and applied to the column. The purified product was recovered by elution under N₂-pressure in 15 ml fractions.

8.1.3. Thin layer chromatography

Qualitative thin layer chromatography (TLC) was performed on pre-coated Merck plastic sheets (silica gel PF₂₅₄, 0.25mm). After development, the plates were sprayed with vanillin-H₂SO₄. R_f values reported are those observed in these qualitative TLC assessments.

Preparative scale thin layer chromatography (PLC) was conducted on glass plates (20 X 20cm) coated with Kieselgel PF254 (1.0 mm), which were air-dried overnight at room temperature. The plates (loaded with 10-15 mg of material per plate) were developed in an appropriate eluent and dried in a stream of air. The bands were distinguished by UV (254 nm) light and scraped off. Compounds were eluted from the adsorbent with acetone, which was removed on a rotary evaporator under reduced pressure at 40°C. Small-scale separations were conducted on Merck pre-coated (0.25) TLC plates, silica gel 60 F₂₅₄ with each plate charged with 3-5 mg of the crude product.

8.2. Spraying reagents

8.2.1. Vanillin-sulphuric acid

The TLC plates were sprayed lightly with a solution of vanillin (1 g) in concentrated sulphuric acid (100 ml) and subsequently heated (*ca* 100 °C) to ensure optimum colour development.

8.2.2. Anisaldehyde

Thin-layer chromatograms were sprayed with a solution of anisaldehyde (5 ml) in concentrated sulphuric acid (5 ml) and ethanol (90 ml) and gently heated to 100 °C to ensure optimum colour development.

8.2.3. Formaldehyde-sulphuric acid¹³⁰

TLC plates were quickly sprayed with 2% (v/v) solution of formaldehyde (37 wt % solution in water) in concentrated sulphuric acid and heated to 120 °C to ensure maximum development of colour.

8.2.4. Bis-diazotized benzidine¹³¹

Benzidine (5 g) dissolved in concentrated hydrochloric acid (14 ml) was added to distilled water (980 ml). The mixture (30 ml) was dissolved in sodium nitrite (10 % m/v, 20 ml). Paper chromatograms were gently sprayed with the freshly prepared mixture of this solution and subsequently washed with for 1 hour under running tap water.

8.3. Chemical methods

8.3.1. Acetylation¹³²

Dry phenolic material was dissolved in a minimum volume of pyridine and twice the amount of acetic anhydride was added. After 8-12 hours at ambient temperatures, crushed ice was added to precipitate the acetylated material which was filtered and excess pyridine washed out with cold water.

8.3.2. Methylation with diazomethane¹³³

Methylations were performed with an excess of diazomethane prepared by the reaction of a cold (-10 °C) potassium hydroxide [5g in ethanol (95%, 55 ml, v/v)] with N-methyl-N-nitroso-*p*-toulene sulphonamide (22 g) in cold ether (150 ml) and distilled directly into a solution of dry phenolic material (250 mg) in methanol (5-10 ml) at -10 °C. After 48 hours at -15 °C the excess diazomethane and solvents were evaporated at room temperature.

¹³⁰H. M. Saayman and D. G. Roux. *Biochem. J.*, **1965**, 96, 36.

¹³¹D. G. Roux and E. A. Maihs. *J. Chromatogr.*, **1960**, 4, 65.

¹³²T. Kametani and S. Kano. *J. Pharmac. Soc. Japan*, **1962**, 82, 1059.

¹³³A. I. Vogel. In *Textbook of Practical Organic Chemistry*, Longmans, London, **1967**, 979.

8.4. Anhydrous solvents and reagents

THF was predried with sodium metal, and was refluxed over sodium/benzophenone under N₂ until a dark blue colour persisted with subsequent fresh distillation under N₂ prior to use.

Acetonitrile was left over 4Å molecular sieves and was refluxed under N₂ for 48 hours with subsequent distillation under N₂ prior to use.

DMF was left over barium oxide for 24 hours. Barium oxide was filtered off and the solvent subsequently refluxed over NaH under N₂ and stored under N₂.

Methanol was left over anhydrous potassium carbonate for 24 hours. The potassium carbonate was filtered off and the solvent subsequently distilled over 3Å molecular sieves and stored under N₂.

8.5. Freeze-drying

Phenolic material in aqueous solution was freeze-dried using a Virtis Freezemodi; 12SL at 40 millitorr.

8.6. Spectroscopical methods

8.6.1. Nuclear magnetic resonance spectroscopy

NMR spectra were recorded on a Bruker AVANCE DPX₃₀₀ spectrometer with tetramethylsilane an internal standard. The solvents used were deuteriochloroform (CDCl₃, δ_H 7.24) and deuterioacetone [(CD₃)₂CO/acetone d₆, δ_H 2.04]. Chemical shifts are reported in parts per million (ppm) on the δ-scale and coupling constants were measured in Hz. Abbreviations are used as follows:

Abbreviation	Signal multiplicity	Abbreviation	Signal multiplicity
s	singlet	dd	doublets of doublets
d	doublet	br	broadened
t	triplet	<i>eq</i>	equatorial
m	multiplet	<i>ax</i>	axial

Table 8.1: Abbreviations used in describing ¹H NMR signal multiplicities.

8.6.2. Circular dichroism

CD spectra were recorded on a Jasco J-710 spectropolarimeter in spectrophotometric grade methanol (~ 1 mg/10 ml MeOH). The formula used to calculate the molecular ellipticity (θ) was:

$$[\theta] = \frac{(L)(scale)(molecular\ weight)(100)}{[length\ of\ tube(cm)][concentration(g/l)]}$$

where L is the difference (at any given wavelength) between the reading (in cm) of the compound in solution and the reading (in cm) of pure solvent.

8.6.3. Mass spectrometry

All fast atom bombardment (FAB) and electron ionisation (EI) mass spectra were recorded on a VG70-70E double-focusing mass spectrometer using a VG-250J data system and an Iontech saddlefield FAB gun. M⁺ denotes the molecular ion.

8.7. Abbreviations

The following abbreviations for solvents are used through out the experimental section:

A	=	acetone
C	=	chloroform
DMF	=	dimethylformamide
E	=	diethyl ether
EA	=	ethyl acetate
EtOH	=	ethanol
H	=	hexane
MeOH	=	methanol
THF	=	tetrahydrofuran
T	=	toluene

CHAPTER 9

9. Isolation of compounds from *Pericopsis elata*

9.1. Enrichment of the heartwood extract

The dried pulverized heartwood (5.06 kg) of *Pericopsis elata* were consecutively extracted with hexane (15 L x 4), acetone (22 L x 4), methanol (20 L x 10) and acetone-water 70/30 (22 L x 4) followed by evaporation under reduced pressure at 40 °C. Yellow residual oil (100.1g), which previously reported as terpenoid was obtained from hexane extract. Acetone and methanol extracts 498.64 g and 652.72 g, respectively, yielded dark brown material.

The acetone (120.14 g) and the methanol (120.01 g) extracts were partitioned in H₂O: *sec*-BuOH: hexane (5:4:1) in a fifteen-tube Craig countercurrent assembly (200 ml of organic and 200 ml of aqueous phase per tube), by ten transfers of the top phase. Following paper chromatographic analysis of the acetone extract, fractions from the Craig were combined as follows:

Fraction	Tubes	Mass (g)
AAP	1 – 3	3.0
ABP	4 – 7	1.36
ACP	8 – 10	1.91
ADP	11 - 15	110.06

Table 9.1: The four fractions of the acetone extract from the Craig countercurrent distribution.

9.2. Isolation of compounds from the acetone extract

Fraction ADP (25.01 g) was dissolved in ethanol and subjected to column chromatography on Sephadex LH-20 (4 x 150 cm column, flow rate 30 ml/32 min) in ethanol to give 10 fractions (A1-A10) after analysis with TLC (T:A:M, 6:3:1, v/v/v).

Fraction	Test tubes	Mass (g)
A1	1-37	1.51
A2	38-97	0.39
A3	98-177	10.0
A4	178-223	1.32
A5	224-367	4.81
A6	368-553	0.30
A7	554-749	2.21
A8	752-833	0.21
A9	836-1133	0.45
A10	1134-1368	0.33

Table 9.2: Fractions obtained from the column separation of fraction ADP

Fractions A1, A5, A6, A9 and A10 are yet to be investigated.

Acetylation of fraction A2 (350 mg) followed by PLC purification (T: A: M, 90: 8: 2) gave six bands (**Table 9.3**).

Bands	Mass (mg)	R _f values
A2.1	49.7	0.65
A2.2	97.2	0.45

Table 9.3: Fractions obtained from the PLC separation of A2.

9.2.1. 4'-Acetoxy-2'-hydroxy-4-methoxy- α -methyldeoxybenzoin (72).

Band A2.1 afforded the title compound as a colourless amorphous solid (R_f 0.65, 49.7 mg).

¹H NMR **Plate 6, Table 5.5**.

9.2.2. (*R*)- α -4,2',4'-Tetraacetoxydihydrochalcone¹³⁴ (71).

Band A2.2 yielded the title compound as a yellow amorphous solid (R_f 0.45, 97.2 mg).

¹H NMR Plate 5, Table 5.4.

Fraction A3 (200 mg) was acetylated and resolved by PLC (T: A: M, 90: 9: 1) into three bands (Table 9.4).

Fraction	Mass (mg)	R_f values
A3.1	16.6	0.55
A3.2	6.4	0.50
A3.2	97.5	0.39

Table 9.5: Fractions obtained from the PLC separation of A3.

Further PLC purification (T: A; EA, 8: 1: 1) of A3.1 afforded 2 bands A3.1.1 and A3.1.2.

9.2.3 *trans*-4,3',5'-Triacetoxystilbene (73).

Band A3.1.1 yielded the title compound as a light yellow amorphous solid (R_f 0.65, 7.9 mg).

¹H NMR Plate 7, Table 5.6

9.2.4 5,7,4'-Triacetoxyisoflavone (69).

Fraction A3.1.2 afforded the title compound as a light yellow amorphous solid (R_f 0.49, 9.7 mg).

¹H NMR Plate 3, Table 5.3

9.2.5 5,7-Diacetoxy-4'-methoxyisoflavone (70).

Fraction A3.2 yielded the title compound as a colourless amorphous solid (R_f 0.50, 6.4 mg).

¹H NMR Plate 4, Table 5.3

9.2.6 5,7,4'-Triacetoxyflavanone (67).

Fraction A3.3 afforded the title compound as a colourless amorphous solid (R_f 0.39, 97.5 mg).

¹H NMR Plate 1, Table 5.1

¹³⁴ F. Ferrari, B. Botta and R. A. De Lima. *Phytochemistry*, **1983**, 22, 1663.

Acetylation followed by PLC purification (T: A: EA, 8: 1: 1) of A4 (50 mg) afforded two bands A4.1 and A4.2.

Band A4.1 (R_f 0.45, 10.5 mg) yielded the same compound as A3.1.1

9.2.7. *trans*-4,3',5'-Triacetoxy-3-methoxystilbene¹³⁵ (75).

Fraction A4.2 afforded the title compound as a light yellow amorphous solid (R_f 0.32, 15.4 mg).

¹H NMR **Plate 10, Table 5.8**

Acetylation of A7 (150 mg) followed by PLC purification (T: A: M, 90: 9: 1) afforded six bands.

Fraction	Mass (mg)	R_f values
A7.1	100.9	0.36
A7.2	19.4	0.25

Table 9.6: Fractions obtained from the PLC separation of A7.

9.2.8. *rel*-2,3-*trans*-2-(3,5-Diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-6-[2-(3,5-diacetoxyphenyl)]-*E*-1-ethenyl]-4-acetoxy-2,3-dihydrobenzofuran (81).

Band 7.5 afforded the title compound as a light yellow amorphous powder (R_f 0.36, 100.9 mg).

¹H NMR **Plate 16, Table 6.6**

9.2.9. *rel*-2,3-*trans*-2-(3,4-Diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-4-[2-(3,4-diacetoxyphenyl)]-*E*-1-ethenyl]-6-acetoxy-2,3-dihydrobenzofuran (78).

Band 7.6 afforded the title compound as a light yellow amorphous solid (R_f 0.25, 19.4 mg).

¹H NMR **Plate 13, Table 6.3**

Fraction A8 (100 mg) was acetylated and resolved by PLC (T: A: EA, 8: 1: 1) into three bands A8.1, A8.2 and A8.3 (**Table 9.6**).

¹³⁵ G. D. Manners and E. P. Swan. *Phytochemistry*, **1971**, *10*, 607.

Fraction	Mass (mg)	R _f values
A8.1	10.4	0.69
A8.2	35.6	0.51
A8.3	45.7	0.42

Table 9.6: Fractions obtained from the PLC separation of A8.

Bands A8.1 yielded the same compound as A4.1 and A8.2 afforded identical compound as A4.2.

9.3. Isolation of compounds from the methanol extract

Methanol extract (30.0g) was dissolved in ethanol and subjected to column chromatography on Sephadex LH-20 (4 x 150 cm column, flow rate 30 ml/32 min) in ethanol to give 11 fractions (B1-B11) after analysis with TLC (T:A:M, 6:3:1, v/v/v).

Fractions	Test tubes	Yields (g)
B1	1-157	0.35
B2	158-189	1.54
B3	190-295	7.32
B4	296-451	8.92
B5	452-681	4.60
B6	682-725	0.46
B7	726-831	1.23
B8	832-983	1.23
B9	982-1192	0.78
B10	1194-1328	0.37
B11	1330-1714	0.88

Table 9.7: Fractions obtained from the column separation of acetone extract.

9.3.1. *trans*-3,4,3',5'-Tetrahydroxystilbene¹³⁶ (48).

Fraction B5 afforded the title compound as a light yellow amorphous solid.

¹H NMR Plate 8, Table 5.7

9.3.2. *trans*-3,4,3',5'-Tetraacetoxystilbene (74).

Acetylation of B5 yielded the title compound as a light yellow amorphous solid.

¹H NMR Plate 9, Table 5.7

Methylation of B7 (100 mg) followed by PLC purification (T: A, 8: 2) yielded five bands (Table 9.8).

Fraction	Mass (mg)	R _f values
B7.1	10.5	0.82
B7.2	9.7	0.71
B7.3	3.5	0.66
B7.4	27.3	0.57
B7.5	2.3	0.49

Table 9.10: Fractions obtained from the PLC separation of B7

9.3.3. 5,7,3',4'-Tetramethoxyflavanone (68).

Band B7.1 afforded the title compound as a yellow amorphous solid (R_f 0.82, 10.5 mg).

¹H NMR Plate 2 Table 5.2

9.3.4. *rel*-2,3-*trans*-2-(3,4-dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-4-[2-(3,5-dimethoxyphenyl)]-Z-1-ethenyl]-6-methoxy-2,3-dihydrobenzofuran (79).

Band B7.2 afforded the title compound as a light yellow amorphous solid (R_f 0.71, 9.7 mg).

¹H NMR Plate 14 Table 6.4

9.3.5. *rel*-2,3-*trans*-2-(3,4-Dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-6-[2-(3,5-dimethoxyphenyl)-E-1-ethenyl]benzodioxane (76).

Band B7.3 afforded the title compound as a light yellow amorphous solid (R_f 0.66, 3.5 mg).

¹H NMR Plate 11, Table 6.1

¹³⁶ W. Grassman and H. Endres. *J. Chem. Soc.*, **1965**, 4579.

9.3.6. *rel-2,3-trans-2-(3,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-4-[2-(3,4-dimethoxyphenyl)]-E-1-ethenyl]-6-methoxy-2,3-dihydrobenzofuran (77).*

Band B7.4 yielded the title compound as a colourless amorphous solid (R_f 0.57, 27.3 mg).

$^1\text{H NMR}$ **Plate 12, Table 6.2**

Further PLC purification (T: A: EA, 7: 2: 1) of B7.5 yielded two bands B7.5.1 (R_f 0.65 1.3 mg) and B7.5.2 (R_f 0.49, 0.9 mg). Band B7.5.1 did not contain the compound pertaining to this investigation.

9.3.7. *rel-2,3-trans-4-Formyl-2-(3,4-dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)- 6-methoxy-2,3-dihydrobenzofuran (80).*

Band B7.5.2 afforded the title compound as a light yellow amorphous solid (R_f 0.49, 0.9 mg).

$^1\text{H NMR}$ **Plate 15, Table 6.5**

10. Synthesis of the monomeric and oligomeric stilbenes

10.1. Formation of the phosphonate salt

10.1.1. 3,5-Dimethoxybenzyl alcohol (**83**)¹³⁷

To the mixture of 3,5-dimethoxybenzaldehyde (**82**) (30.1 mmol, 5.0g) in EtOH (20 ml), NaBH₄ (10.03 mmol, 379.6 mg) was added with constant stirring at room temperature for 30min. Addition of cold H₂O and acidification by 1M HCl (1ml) followed by extraction of the reaction mixture with EtOAc (3 x 30 ml) gave the crude product after drying (Na₂SO₄) and removal of the solvent. The product 3,5-dimethoxybenzyl alcohol (**83**) was collected as yellow crystals (4.78 g, 96%) M.p. (46-49 °C).

¹H NMR (CDCl₃) (**Plate 17**) (**85**): δ 6.53 (d, *J* 2.5 Hz, H-2,6), δ 6.39 (t, *J* 2.5 Hz, H-4), δ 4.64 (s, *J* 1.0 Hz, CH₂), δ 3.57 (s, 2 x OCH₃).

10.1.2. 3,5-Dimethoxybenzyl bromide (**84**)¹³⁸

PBr₃ (1.2 eq) was mixed with a solution of 3,5-dimethoxybenzyl alcohol (**83**) (14.9 mmol, 2.5 g) in dry THF (20 ml) and the mixture stirred for 1h at room temperature. The product 3,5-dimethoxybenzyl bromide (**84**) was collected as colourless crystals after crystallization from hexane (2.45 g, 98%) M.p. 69-71°C.

¹H NMR (CDCl₃) (**Plate 14**) (**84**): δ 6.57 (d, *J* 2.5 Hz, H-2,6), δ 6.42 (t, *J* 2.5 Hz, H-4), δ 4.44 (s, *J* 1.0 Hz, CH₂), δ 3.81 (s, 2 x OCH₃).

¹³⁷ S. J. Garden, M. B. Côrrea and A. C. Pinto. *Tetrahedron Lett.*, **2003**, *44*, 7617.

¹³⁸ C. R. Noller and R. Dinsmore. *Org. Syn. Coll.*, **1943**, *2*, 358.

10.1.3 3,5-Dimethoxy-triphenyl-benzylphosphonate (salt) (85)¹⁰⁹

To a solution of 3,5-dimethoxybenzyl bromide (**84**) (8.7 mmol, 2.0 g) in dry acetonitrile, triphenylphosphine (0.22 mmol, 56.7 mg) was added and the mixture was refluxed for 1h. The cooled mixture was filtered and the precipitate dried. The product was collected as a white powder (50%).

¹H NMR (CDCl₃) (**Plate 19**) (**85**): δ 7.87 (m, *J* 2.5 Hz, 3 x C₆H₅), δ 6.37 (d, *J* 2.5 Hz, H-2,6), δ 6.41 (t, *J* 2.5 Hz, H-4), δ 5.29 (d, *J* 1.0 Hz, CH₂), δ 3.76 (s, 2 x OCH₃).

10.2. Synthesis of monomeric stilbene (87)¹⁰⁹

A mixture of NaOMe (4.2 mmol, 0.28 ml) and 3,5-dimethoxy-diphenyl-benzylphosphonate (**85**) (4.7 mmol, 1.98 g) in dry DMF (20 ml) was stirred for 30 min at 0 °C. 3,4-dimethoxybenzaldehyde (**86**) was added to the reaction mixture and refluxed for 2h. The reaction mixture was allowed to stand overnight at room temperature. A quantity of H₂O (20 ml) was added, the mixture was extracted with ether (3 x 30 ml) and dried with MgSO₄. Evaporation of the solvent followed by PLC (*R_f* 0.57 T: A, 9: 1) gave the methylated *trans*-stilbene (**87**) in 40% yield.

¹H NMR (CDCl₃) (**Plate 20**) (**87**): δ 7.07 (dd, *J* 8.5, 2.5 Hz, H-6), δ 7.06 (d, *J* 16.0 Hz, H-α), δ 7.04 (d, *J* 8.5 Hz, H-2), δ 6.93 (d, *J* 16.0 Hz, H-β), δ 6.87 (d, *J* 8.5 Hz, H-5), δ 6.68 (d, *J* 2.5 Hz, H-2',6'), δ 6.41 (t, *J* 2.5 Hz, H-4'), δ 3.97 (s, OCH₃) δ 3.93 (s, OCH₃) δ 3.85 (s, 2 x OCH₃).

10.2.1. Demethylation of monomeric stilbene¹³⁹

Ethaneithiol (1.3 mmol, 0.062 mg in dry DMF (20 ml) was added to the suspension of sodium hydride (of a 50% oil dispersion) in dry DMF under nitrogen and stirred for 5 min. The methylated stilbene (0.41 mmol, 0.1g) in dry DMF was added and the reaction mixture was refluxed for 3 h. The cooled mixture was extracted with ether and evaporated under pressure to give the demethylated stilbene (piceatannol) **48** in 70% yield.

¹H NMR data is the same as that of **48**.

¹³⁹ G. I. Feutrill and R. N. Mirrington. *Tetrahedron Lett.*, **1970**, *11*, 1327.

10.3. Synthesis of dimeric stilbenes (**88** and **89**)¹⁴⁰

A mixture of AgOAc (0.21 mmol, 34.2 mg) and piceatannol (**48**) (0.21 mmol, 50.0 mg) in dry MeOH was heated for 1 hour at 60 °C. The reaction mixture turned to an ash colour due to the formation of the silver mirror on the inside of the flask. Removal of the solvent under reduced pressure followed by PLC separation (T: A, 5: 5) afforded two compounds, **88** (R_f 0.33, 30.2mg, 30.3%) and **89** (R_f 0.22, 15.5 mg, 15.0%).

¹H NMR (CDCl₃) (**Plate 21**) (**88**): δ 8.5 (s, 6 x OH) 7.18 (d, J 2.5 Hz, H-5), 7.12 (dd, J 8.5, 2.5 Hz, H-7), δ 7.05 (d, J 16.0 Hz, H- α), δ 6.96 (d, J 16.0 Hz, H- β), δ 6.95 (d, J 8.5 Hz, H-8), δ 6.81 (d, J 2.5 Hz H-2'), δ 6.72 (d, J 8.5 Hz, H-5'), δ 6.58 (dd, J 8.5, 2.5 Hz, H-6'), δ 6.57 (d, J 2.5 Hz, H-2'',6''), 6.29 (t, J 2.5 Hz, H-4''), δ 6.25 (d, 2.5 Hz, H-2''',6'''), 6.25 (t, J 2.5 Hz, H-4'''), δ 4.86 (s, H-2,3).

10.3.1. Acetylation and methylation of dimeric stilbenes (**88** and **89**)

10.3.3.1. Methylation of **88**¹²⁵

Methylation of **88** was performed according to the procedure on page 78. The crude product was purified by PLC separation (R_f 0.55, T: A, 8:2) and afforded **76** in 80% yield.

¹H NMR data is the same as that of **76**.

10.3.1.2. Acetylation of **89**¹²⁴

Acetylation of **89** was performed according to the acetylation procedure on page 78. Further PLC purification (R_f 0.67, T: A, 9:1) afforded **81** in 60% yield.

¹H NMR data is the same as that of **81**.

¹⁴⁰ M. Sako, H. Hosakawa, T. Ito and M. Iinuma. *J. Org. Chem.*, **2004**, *69*, 2598.

Summary

Key words: Isolation, structural elucidation, *Pericopsis elata*, flavonoids, isoflavonoids, antioxidants, phytoalexins, stilbenes, dimers, synthesis.

The bark of *Pericopsis elata* (*Afrormosia elata*) is used by the local population of the Democratic Republic of Congo for the treatment of cancer and for external applications because of its weather resistance. Previous phytochemical studies on *Pericopsis elata* resulted in the isolation of flavonoids, isoflavonoids, chalcones and stilbene monomers. Among these, stilbenes are known to have biological activities such as antioxidants, antifungal, and act as microbial inhibitors. They possess COX-1 and COX-2 inhibitory effects, affect lipid peroxidation, LDL oxidation, and function as phytoalexins among other activities.

Prompted by these claims, we conducted an in depth investigation of the heartwood of *P. elata* by extraction, isolation and structural elucidation of the metabolites. The enrichment and fractionation of monomeric and dimeric constituents were accomplished mainly by Craig countercurrent distribution techniques and Sephadex LH-20 gel chromatography. Pure compounds were obtained by derivatization and preparative thin layer chromatography. Structural elucidation of the phenolics is based mainly on NMR spectroscopic methods (¹H NMR, ¹³C NMR, COSY, NOESY, HMBC and HMQC), Mass spectrometry and synthetic methods.

The monomeric compounds isolated during this study comprise the flavanones (naringenin and eriodictyol), the isoflavones (genistein and biochanin A), a dihydrochalcone [(*R*)- α ,4,2',4'-tetraacetoxydihydrochalcone] and a single α -methyldeoxybenzoin (angolensin). Among the stilbene monomers encountered are resveratrol, isorhapontigenin and large amounts of piceatannol.

Structures of the dimeric stilbenes isolated apparently originate from different combinations of piceatannol (3,4,3',5'-tetrahydroxystilbene) (the major constituent ~ 16%

of the phenolic content of *P. elata*) and 3,4,3',4'-tetrahydroxystilbene. To the best of our knowledge *rel-2,3-trans-2-(3,4-dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-6-[2-(3,5-dimethoxyphenyl)-E-1-ethenyl]benzodioxane*, *rel-2,3-trans-2-(3,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-4-[2-(3,4-dimethoxyphenyl)-E-1-ethenyl]-6-methoxy-2,3-dihydrobenzofuran*, *rel-2,3-trans-2-(3,4-diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-4-[2-(3,4-diacetoxyphenyl)-E-1-ethenyl]-6-acetoxy-2,3-dihydrobenzofuran*, *rel-2,3-trans-2-(3,4-dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-4-[2-(3,5-dimethoxyphenyl)-Z-1-ethenyl]-6-methoxy-2,3-dihydrobenzofuran*, *rel-2,3-trans-4-Formyl-2-(3,4-dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-6-methoxy-2,3-dihydrobenzofuran* and *rel-2,3-trans-2-(3,5-diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-6-[2-(3,5-diacetoxyphenyl)-E-1-ethenyl]-4-acetoxy-2,3-dihydrobenzofuran* are novel compounds.

Structural confirmation of the six novel dimeric stilbenes in particular required definition of structure *via* synthetic methods. A synthetic approach was thus developed. This protocol comprises the synthesis of piceatannol *via* the Wittig reaction followed by oxidative coupling of two piceatannols to afford novel dimeric stilbenes. ¹H NMR of the methoxy-derivative of synthetic compounds is identical to that of the derivative of two dimeric stilbenes isolated from *P. elata*.

Opsomming

Sleutelwoorde: Isolasië, struktuurbevestiging, *Pericopsis elata*, flavonoïede, isoflavonoïede, antioksidante, fitoaleksiene, stilbene, dimere, sintese.

Die bas van *Pericopsis elata* (*Afromorsia elata*) word deur die inheemse bevolking van die Demokratiese Republiek van die Kongo vir die behandeling van kanker gebruik en, danksy die weerbestandheid daarvan, ook vir eksterne aanwending. Tydens vorige fitochemiese studies van *Pericopsis elata* is flavonoïede, isoflavonoïede, chalkone en stilbeenmonomere geïsoleer, waar die bewese biologiese aktiwiteite van laasgenoemde antioksidatiewe, antifungale en mikrobiële eienskappe insluit. Stilbene inhibeer COX-1 en COX-2, beïnvloed lipiedperoksidasië, LDL-oksidasië, en tree ook as fitoaleksiene op.

Op grond van hierdie aanspraak het ons 'n omvattende ondersoek na die bas van *P. elata* geloods deur ekstraksië, isolasië en struktuurbevestiging van die metaboliëte. Die verryking en fraksionering van monomeriese en dimeriese komponente is hoofsaaklik deur Craig teenstroomverspreidingsmetode en Sephadex LH-20 jel chromatografie bewerkstellig. Suiwer verbinding is ná derivatisering en preparatiewe dunlaagchromatografie geïsoleer. Struktuurbevestiging van die fenole berus hoofsaaklik op KMR spektroskopiese metode (¹H KMR, ¹³C KMR, COSY, NOESY, HMBC en HMQC), massaspektrometrie en sintetiese metode.

Die monomeriese verbinding wat tydens hierdie studie geïsoleer is, sluit die flavanone (naringenien en eriodiktiol), isoflavone (genisteïen en biochanien A) 'n dihidrochalkoon [(R)- α -?,4,2',4'-tetra-asetoksidihidrochalkoon] en 'n enkele α -metieldeoksibensoïen (angolensien), in. Stilbeenmonomere wat geïsoleer is sluit resveratrol, isorapontigenien en groot hoeveelhede pikeatannol in.

Die strukture van die dimeriese stilbene wat geïsoleer is, spruit oënskynlik uit verskillende kombinasies van pikeatannol (3,4,3',5'-tetrahidroksistilbeen) (die hoofkomponent ~ 16% van die fenoliese inhoud van *P. elata*) en 3,4,3',4'-

tetrahidroksistilbeen. Sover bekend is *rel-2,3-trans-2-(3,4-dimetoksifeniel)-3-(3,5-dimetoksifeniel)-6-[2-(3,5-dimetoksifeniel)-E-1-eteniel]*bensodioksaan, *rel-2,3-trans-2-(3,4-dimetoksifeniel)-3-(3,4-dimetoksifeniel)-4-[2-(3,4-dimetoksifeniel)-E-1-eteniel]-6-metoksi-2,3-dihidrobensofuraan*, *rel-2,3-trans-2-(3,4-diasetoksifeniel)-3-(3,5-diasetoksifeniel)-4-[2-(3,4-diasetoksifeniel)-E-1-eteniel]-6-asetoksi-2,3-dihidrobensofuraan*, *rel-2,3-trans-2-(3,4-dimetoksifeniel)-3-(3,5-dimetoksifeniel)-4-[2-(3,5-dimetoksifeniel))-Z-1-eteniel]-6-metoksi-2,3-dihidrobensofuraan*, *rel-2,3-trans-4-formiel-2-(3,4-dimetoksifeniel)-3-(3,4-dimetoksifeniel)-6-metoksi-2,3-dihidrobensofuraan* en *rel-2,3-trans-2-(3,5-diasetoksifeniel)-3-(3,5-diasetoksifeniel)-6-[2-(3,5-diasetoksi-feniel)-E-1-eteniel]-4-asetoksi-2,3-dihidrobensofuraan* nuwe verbindings.

Die bevestiging van die strukture van veral die ses nuwe dimeriese stilbene, het sintese genoodsaak. 'n Protokol vir sintese is dus ontwikkel. Laasgenoemde behels die sintese van pikeatannol *via* die Wittig-reaksie, gevolg deur die oksidatiewe koppeling van twee pikeatannole om nuwe dimeriese stilbene. ¹H KMR van die metoksi-derivatiewe van gesintetiseerde verbindings is identies aan dié van die derivatiewe van twee dimeriese stilbene geïsoleer uit *P. elata*.

Opsomming

Sleutelwoorde: Isolasië, struktuurbeoordeling, *Pericopsis elata*, flavonoïede, isoflavonoïede, antioksidante, fitoaleksiene, stilbene, dimere, sintese.

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Op grond van hierdie aanspraak het ons 'n omvattende ondersoek na die bas van *P. elata* geloods deur ekstraksië, isolasië en struktuurbeoordeling van die metaboliete. Die verryking en fraksionering van monomeriese en dimeriese komponente is hoofsaaklik deur Craig teenstroomverspreidingsmetode en Sephadex LH-20 jel chromatografie bewerkstellig. Suiwer verbinding is ná derivatisering en preparatiewe dunlaagchromatografie geïsoleer. Struktuurbeoordeling van die fenole berus hoofsaaklik op KMR spektroskopiese metodes (¹H KMR, ¹³C KMR, COSY, NOESY, HMBC en HMQC), massaspektrometrie en sintetiese metodes.

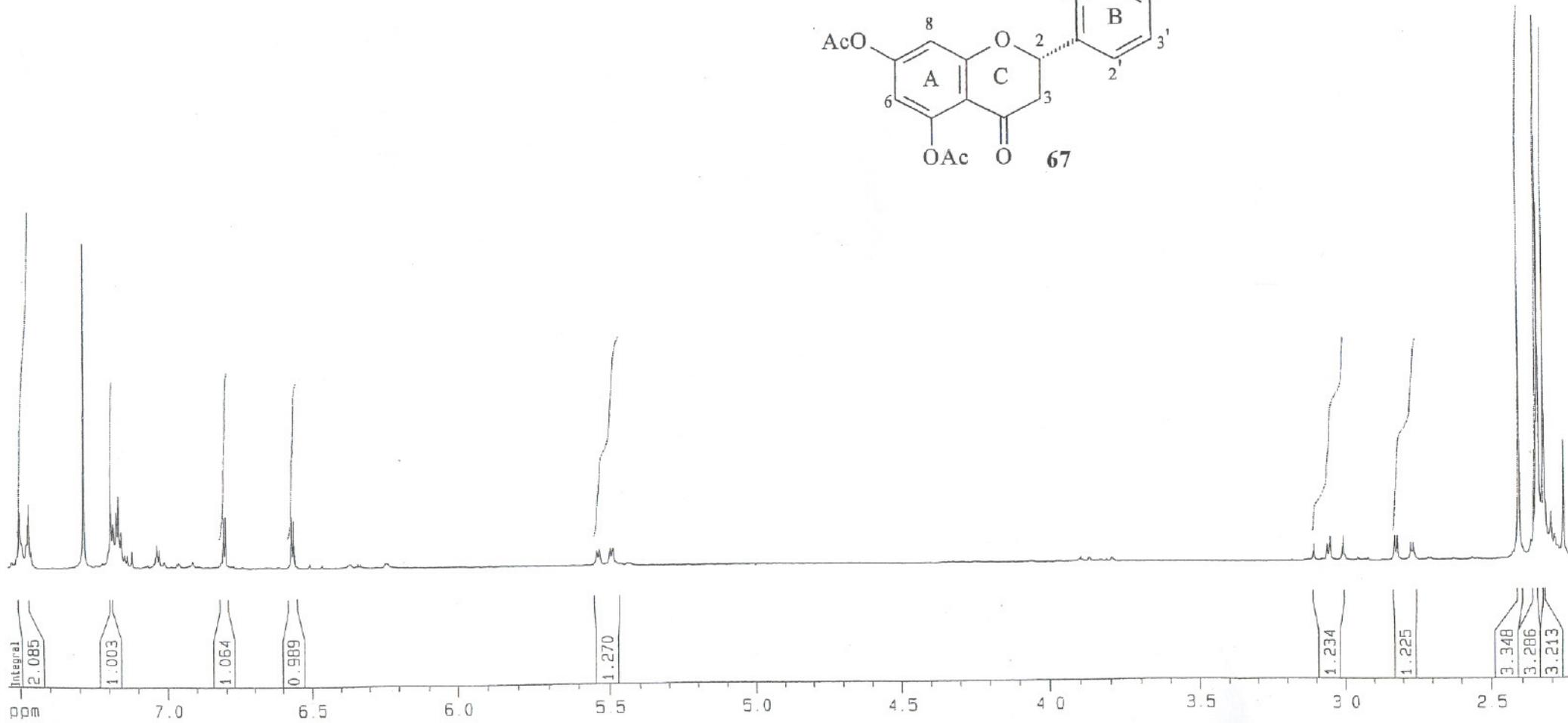
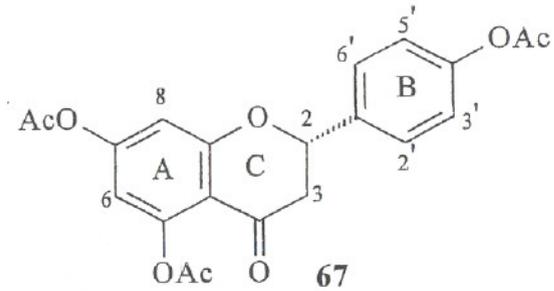
Die monomeriese verbinding wat tydens hierdie studie geïsoleer is, sluit die flavanone (naringenien en eriodiktiol), isoflavone (genisteïen en biochanien A) 'n dihidrochalkoon [(R)- α -?,4,2',4'-tetra-asetoksidihidrochalkoon] en 'n enkele α -metieldeoksibensoïen (angolensien), in. Stilbeenmonomere wat geïsoleer is sluit resveratrol, isorapontigenien en groot hoeveelhede pikeatannol in.

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Die bevestiging van die strukture van veral die ses nuwe dimeriese stilbene, het sintese genoodsaak. 'n Protokol vir sintese is dus ontwikkel. Laasgenoemde behels die sintese van pikeatannol *via* die Wittig-reaksie, gevolg deur die oksidatiewe koppeling van twee pikeatannole om nuwe dimeriese stilbene in goeie opbrengs te lewer. ¹H KMR van die gesintetiseerde verbindings is identies aan dié van die twee dimeriese stilbene geïsoleer uit *P. elata*.

Plate 1: ^1H NMR data of 5,7,4'-triacetoxyflavanone (**67**) [CDCl_3]



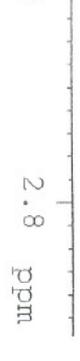
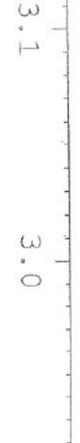
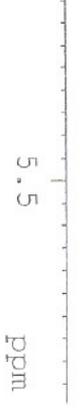
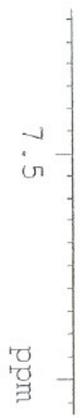
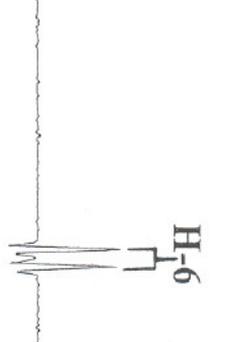
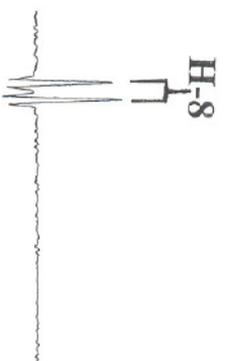
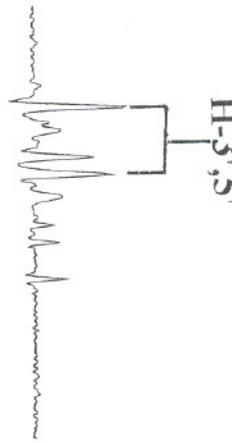
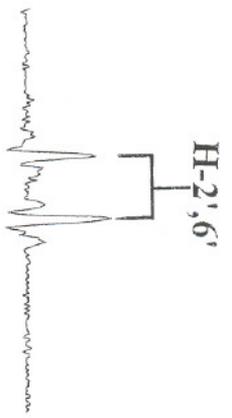
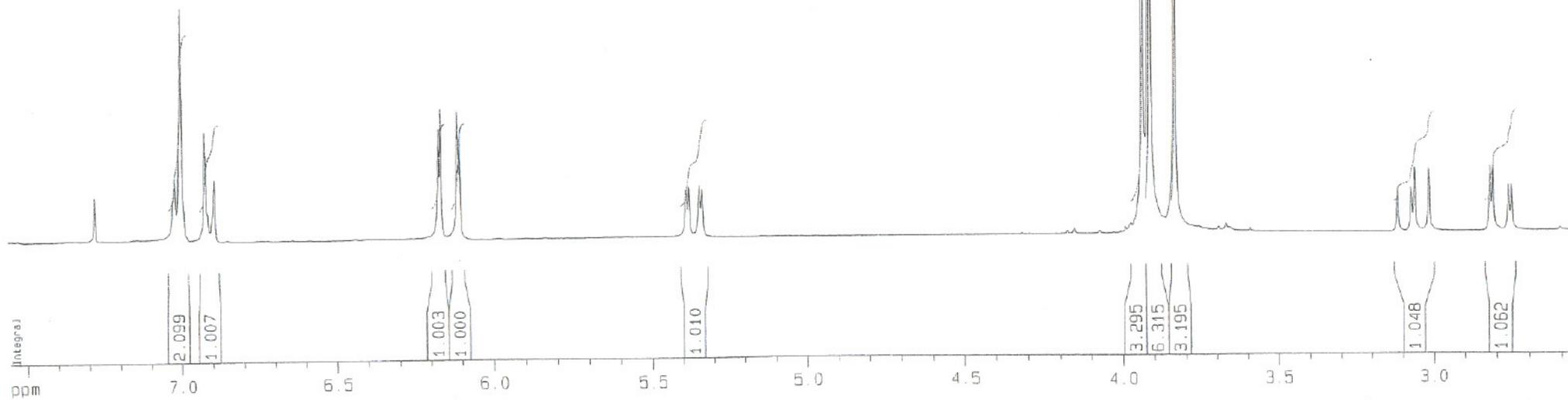
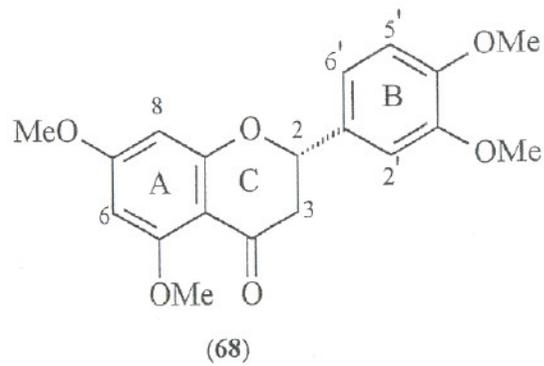
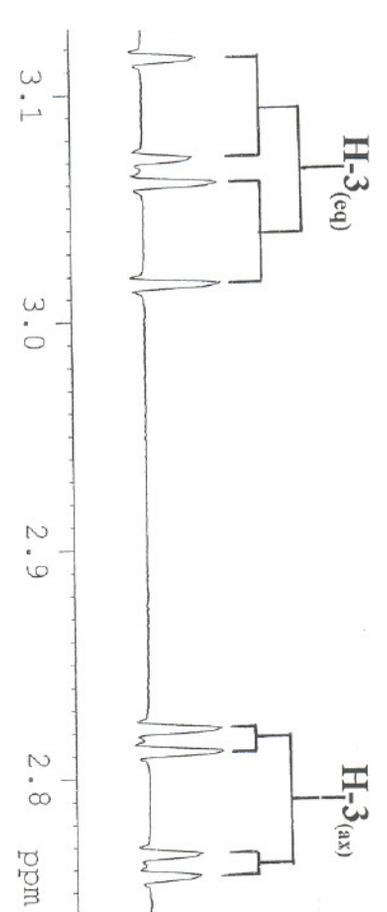
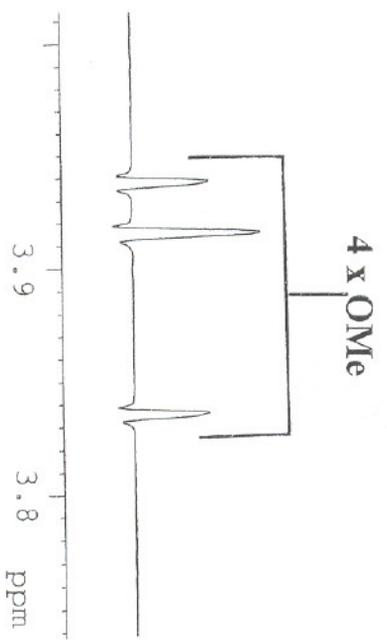
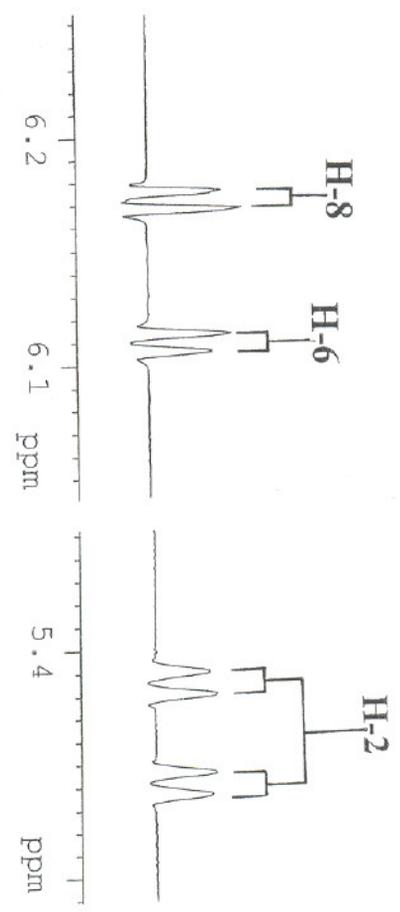
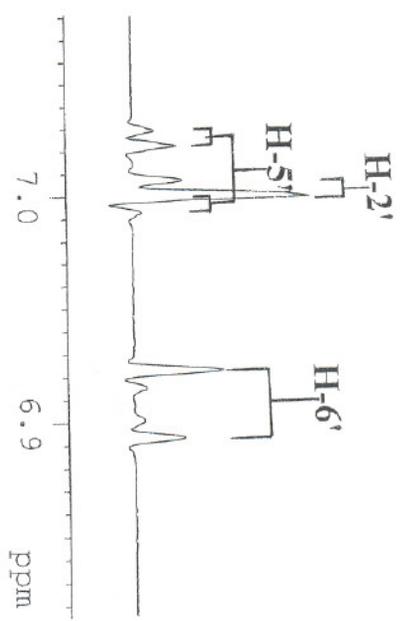
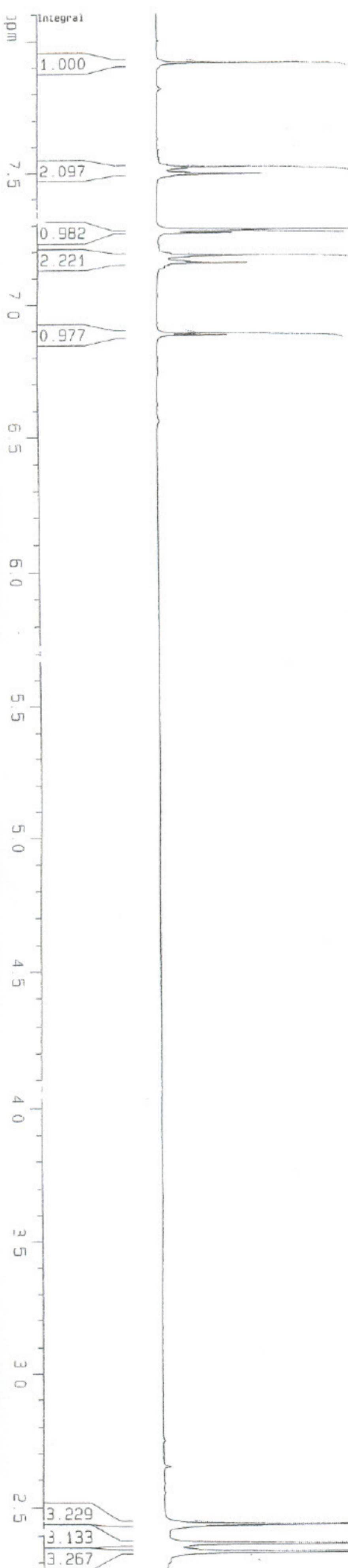


Plate 2: ^1H NMR of 5,7,3',4'-tetramethoxyflavanone (68) [CDCl_3]







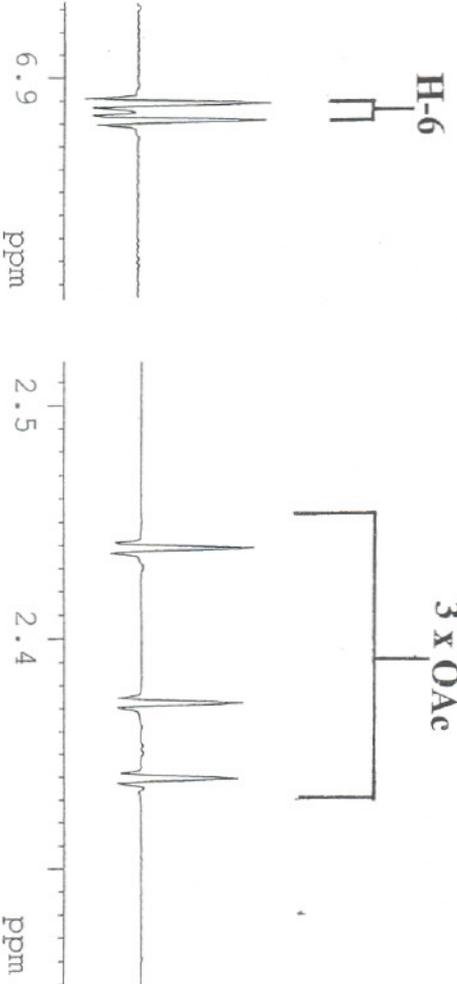
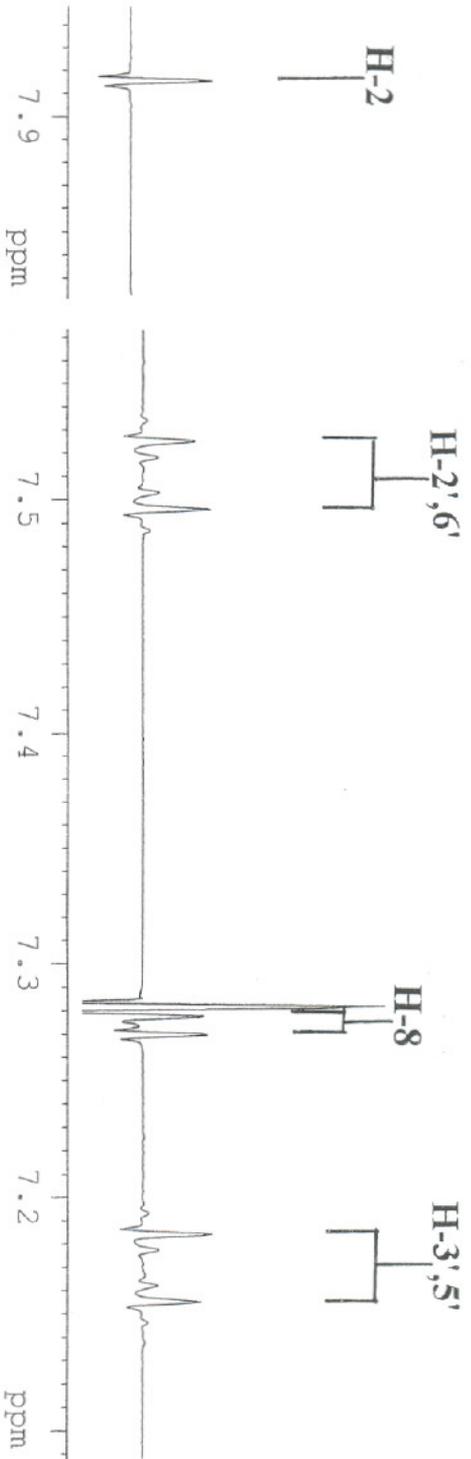
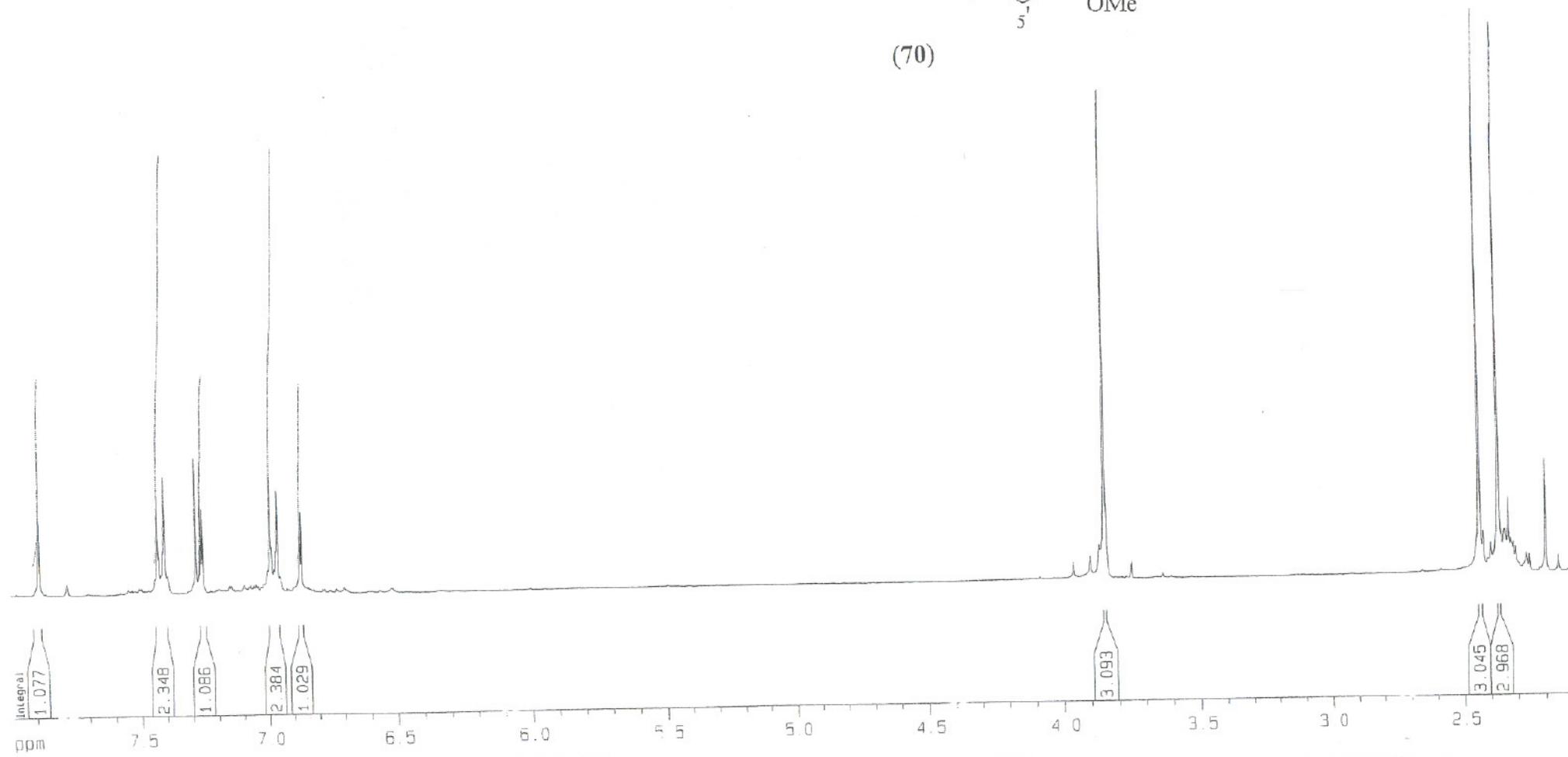
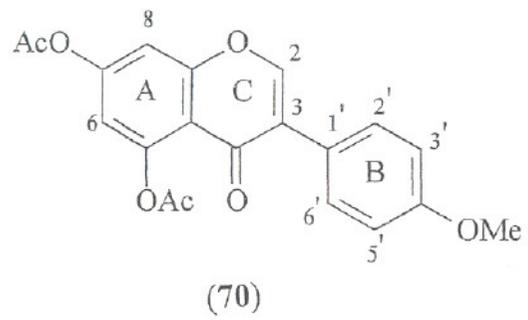


Plate 4: ^1H NMR of 5,7-diacetoxy-4'-methoxyisoflavone (70) [CDCl_3]



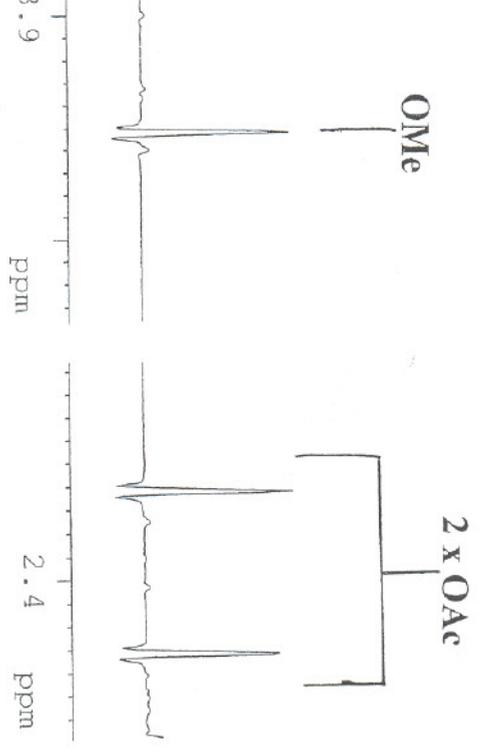
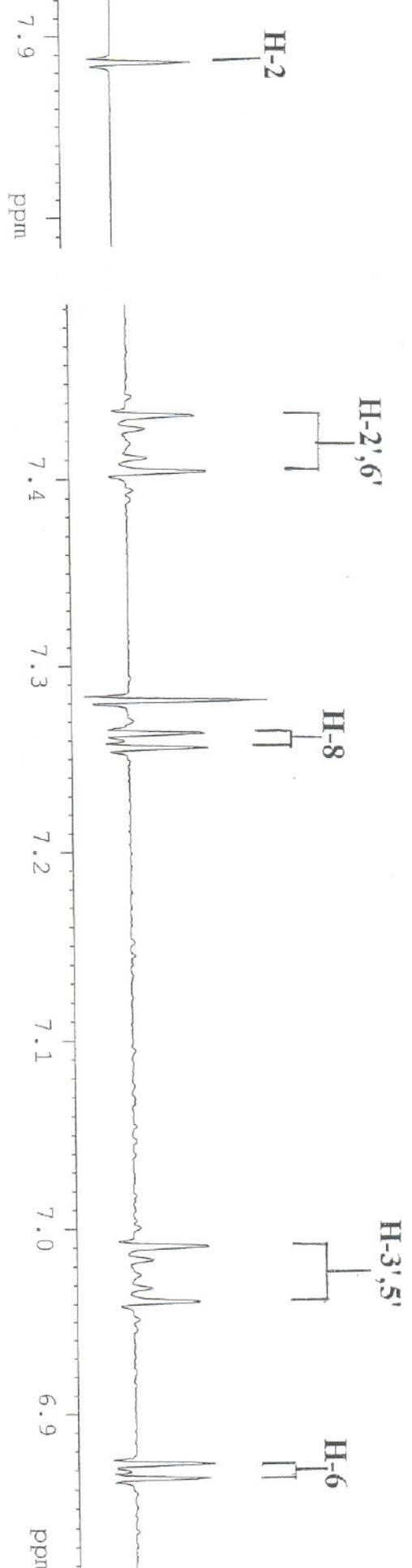
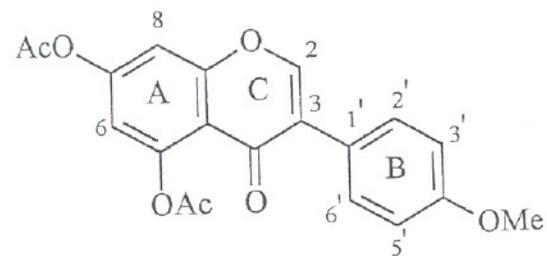
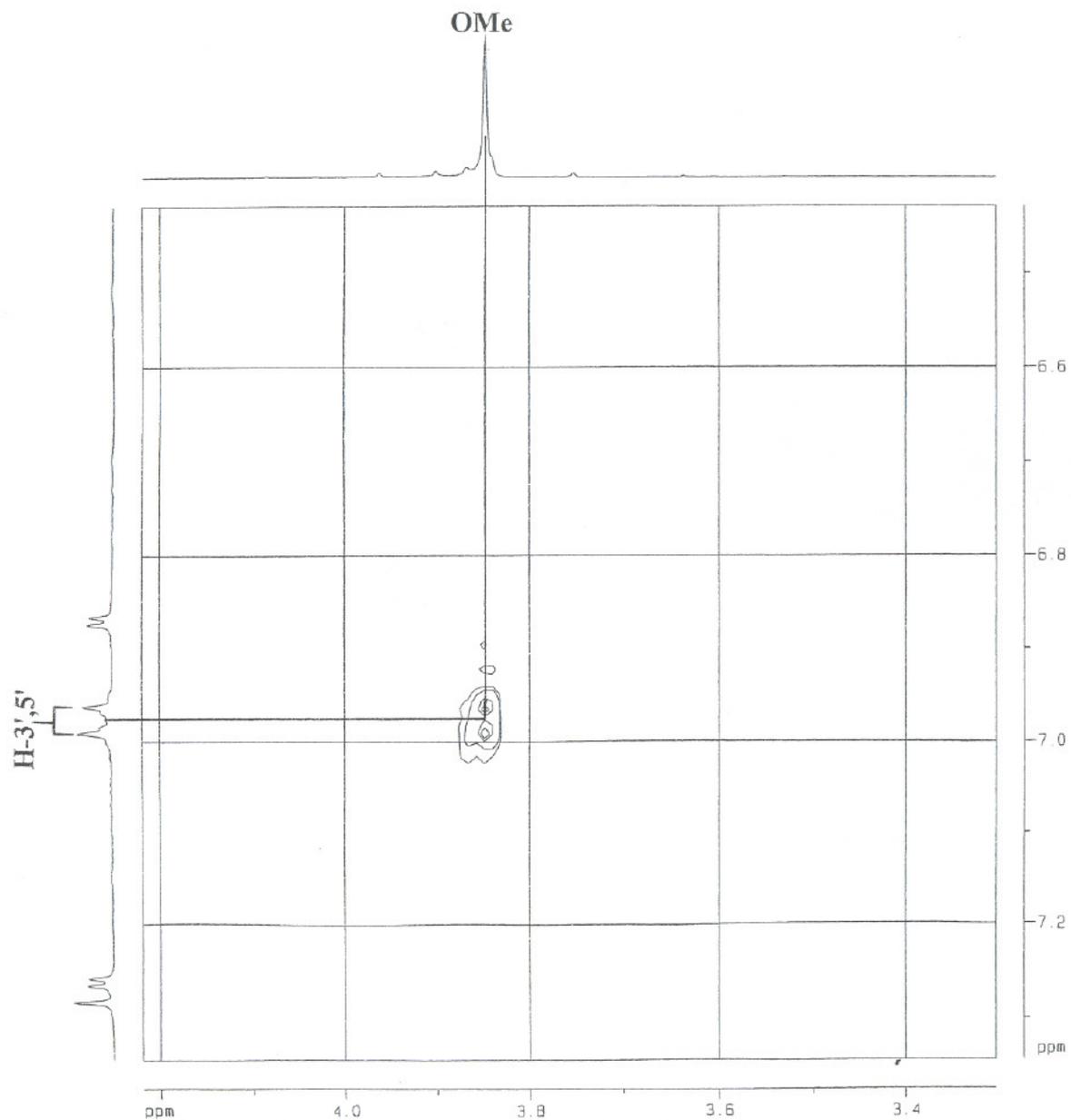
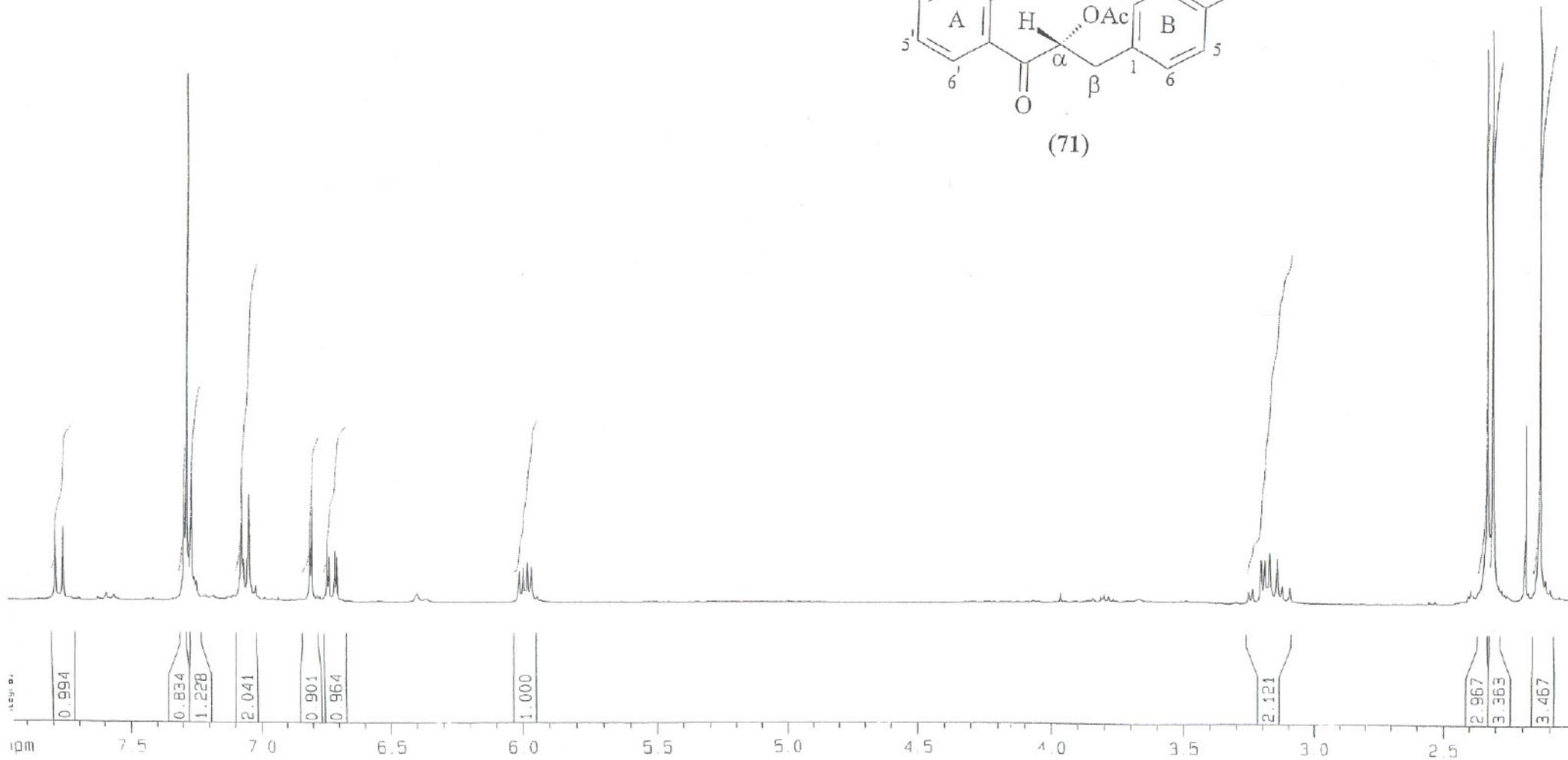
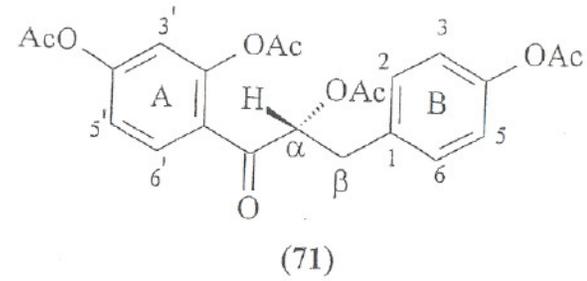


Plate 4a: NOESY of 5,7-diacetoxy-4'-methoxyisoflavone (70) [CDCl₃]



(70)

Plate 5: ^1H NMR of (*R*)- α -4,2',4'-tetraacetoxydihydrochalcone (71) [CDCl_3]



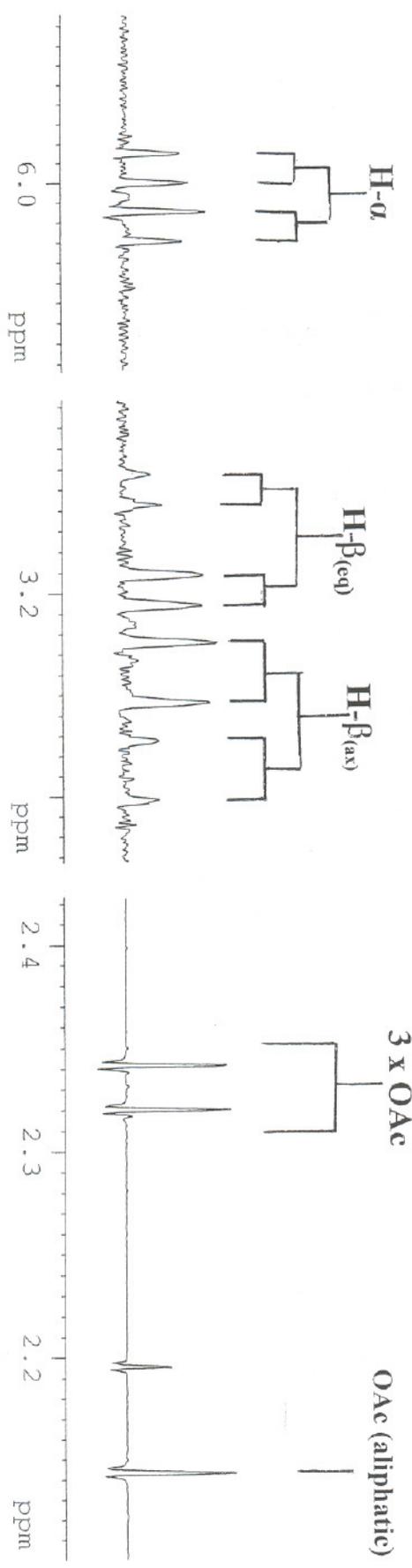
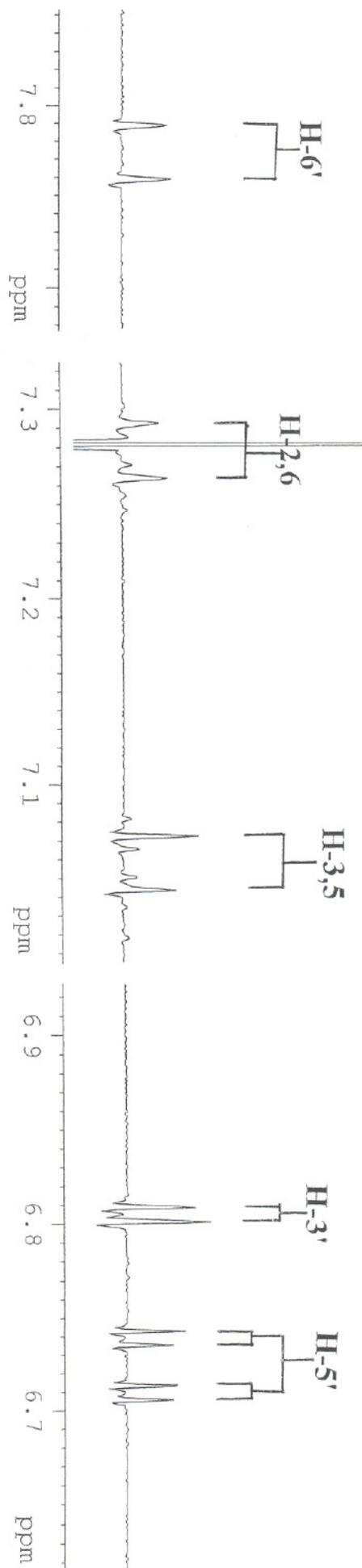
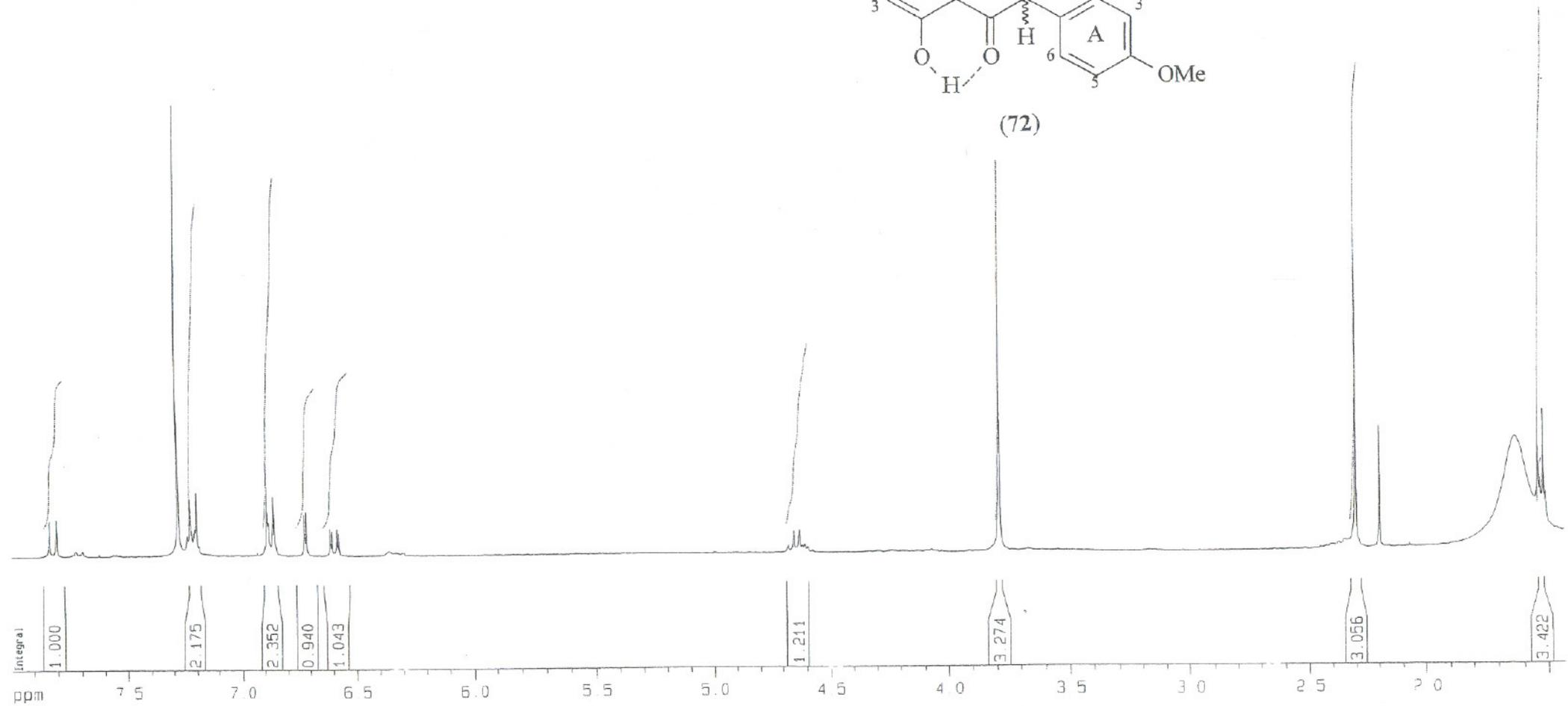
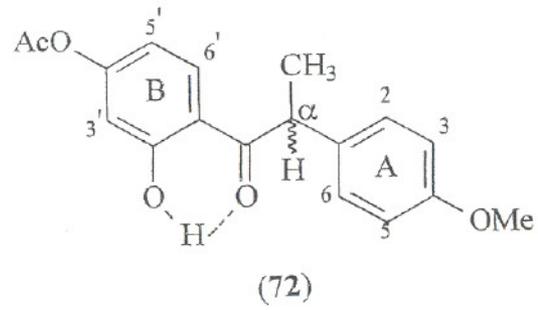


Plate 6: ^1H NMR of 4'-acetoxy-2'-hydroxy-4-methoxy- α -methyldeoxybenzoin (72)
[CDCl₃]



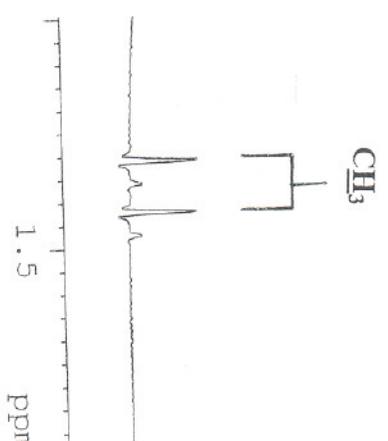
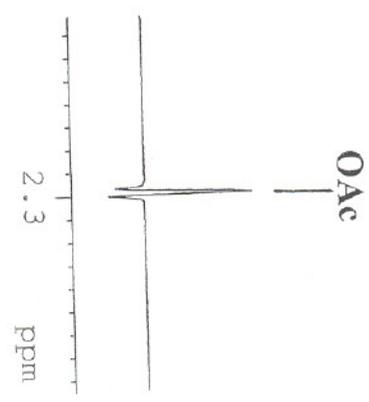
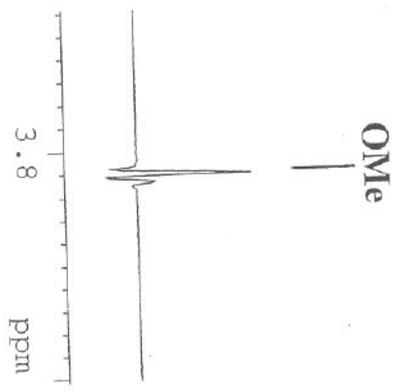
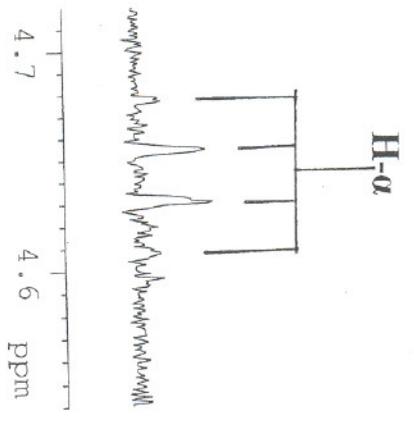
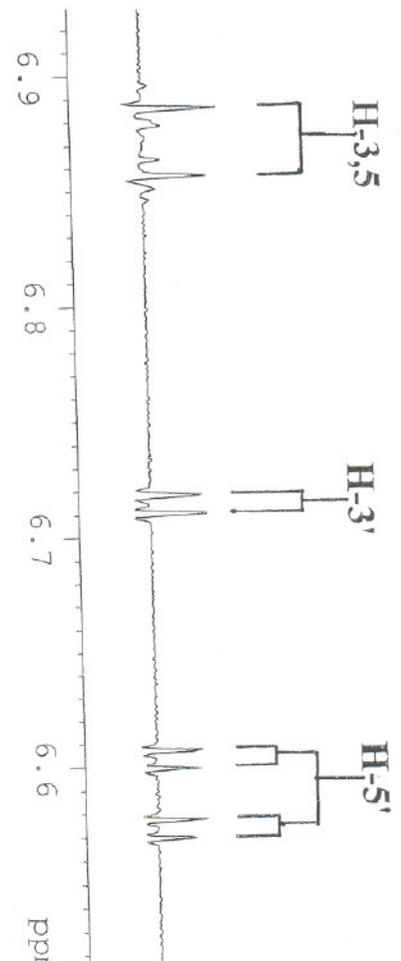
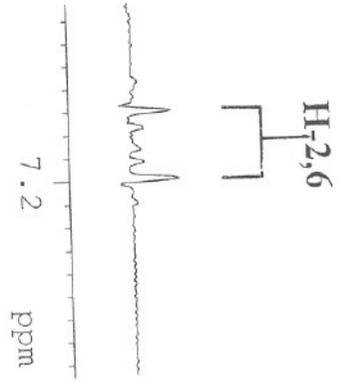
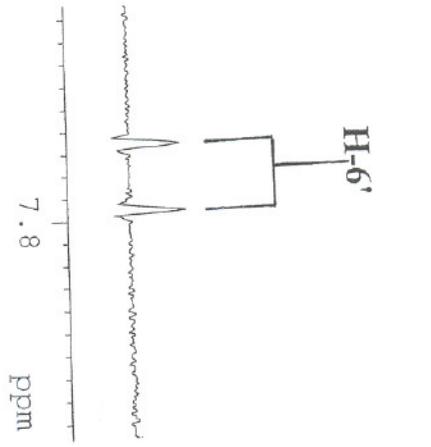


Plate 6a: NOESY of 4'-acetoxy-2'-hydroxy-4-methoxy- α -methyldeoxybenzoin (72)
[CDCl₃]

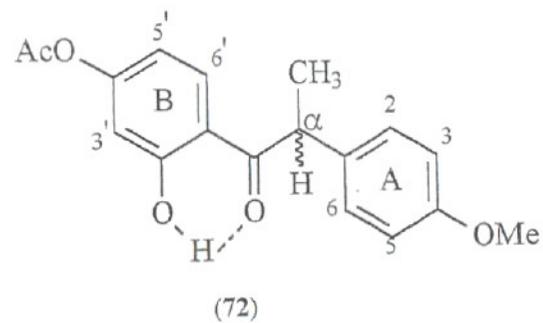
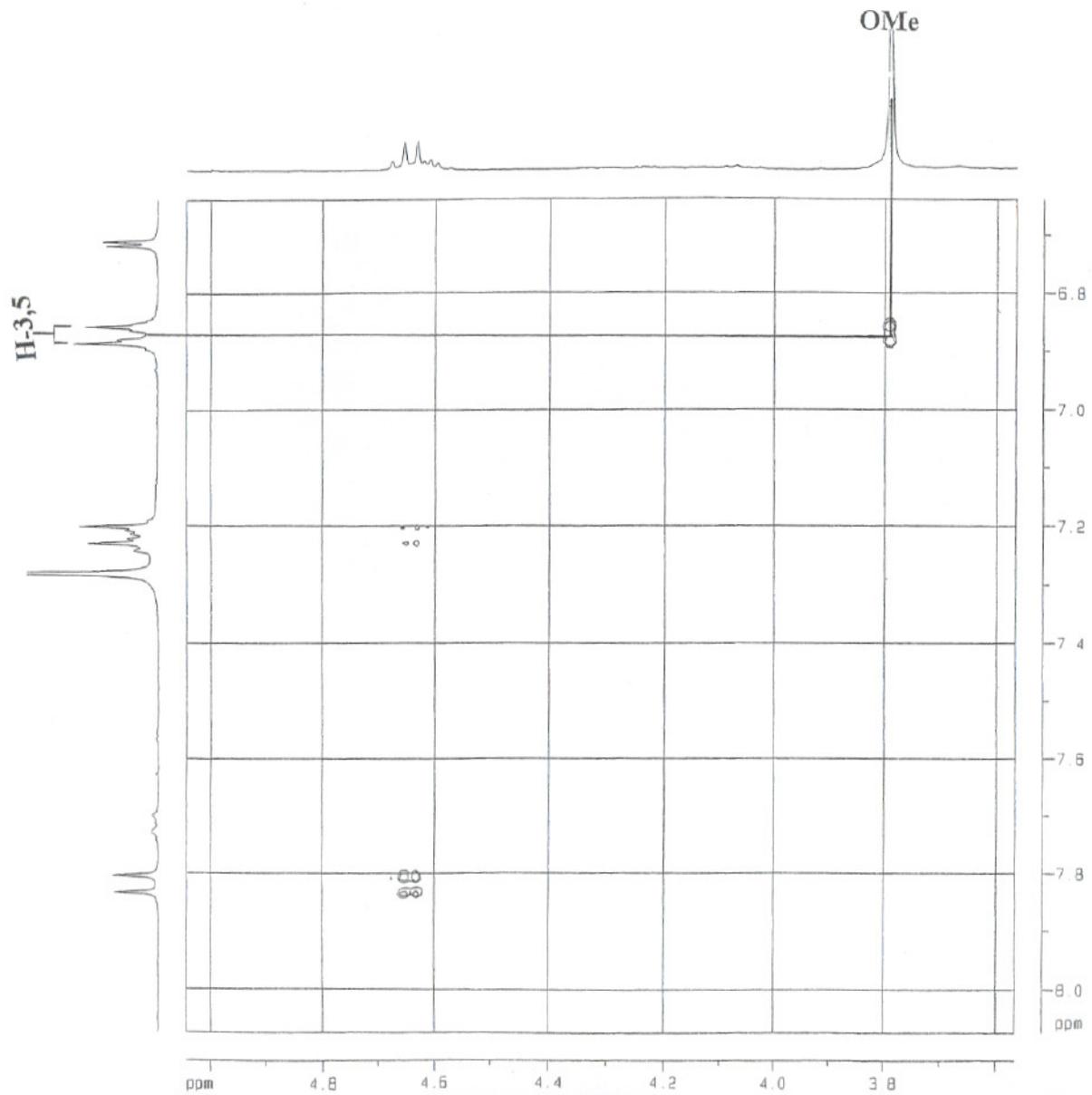
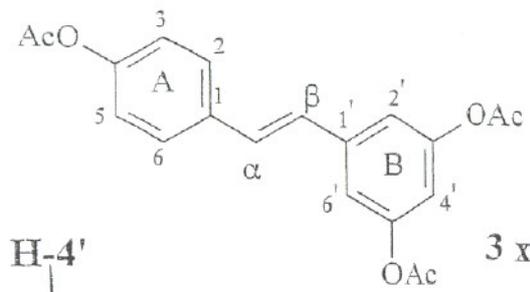


Plate 7: ^1H NMR data of *trans*-4,3',5'-triacetoxystilbene (**73**) [CDCl_3]



H-2,6

H-2',6'

H-3,5

H- α

H- β

H-4'

3 x OAc

7.5 ppm

7.2

7.1

7.0

6.9

6.8 ppm

2.4

ppm

Integral

1.009

3.957

1.057

1.000

ppm

7.0

6.5

6.0

5.5

5.0

4.5

4.0

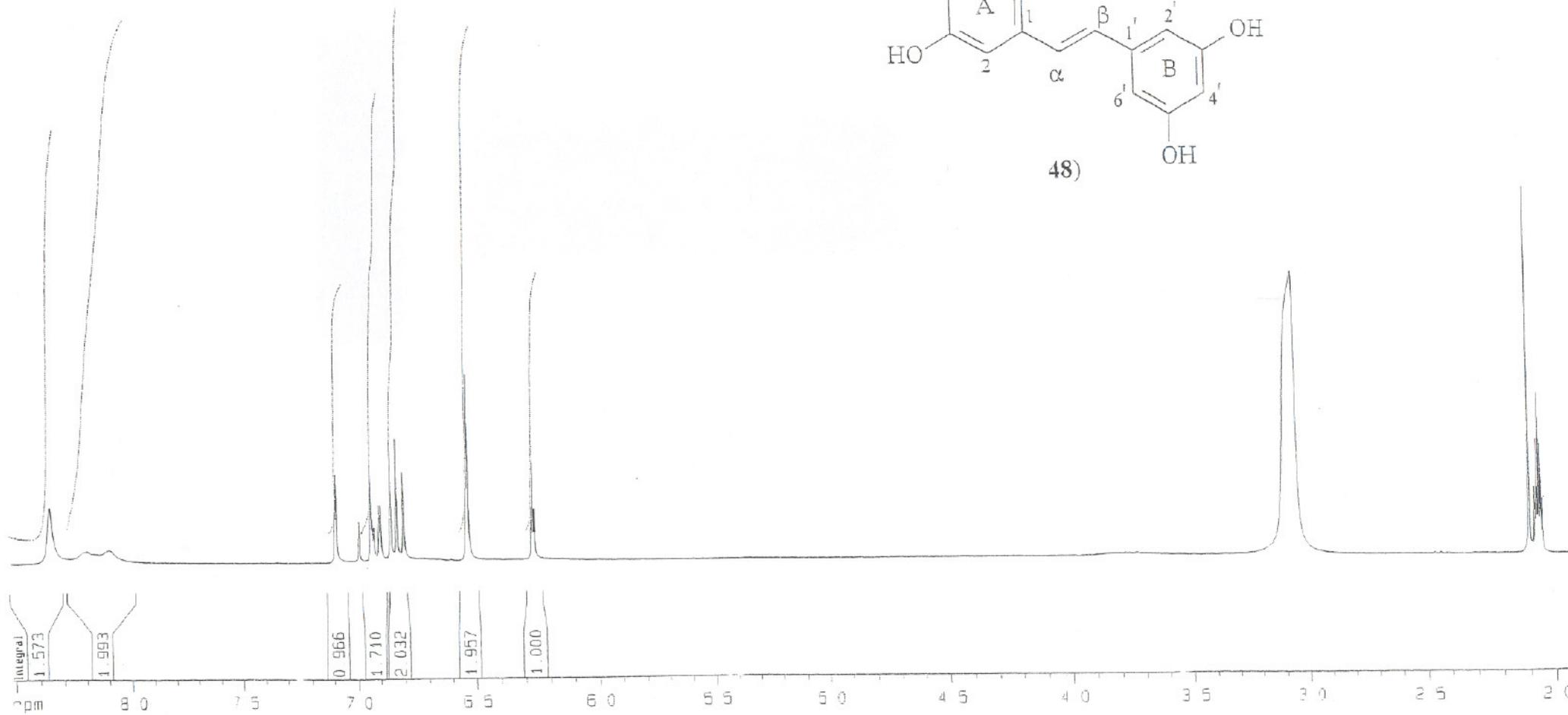
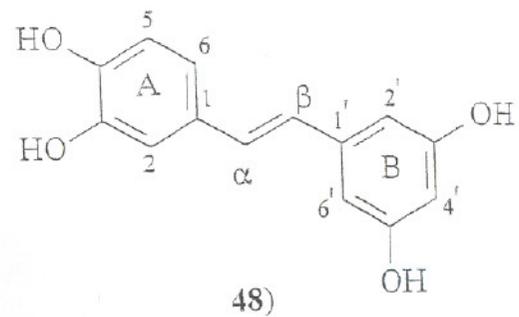
3.5

3.0

2.5

8.957

Plate 8: ^1H NMR data of *trans*-3,4,3',5'-tetrahydroxystilbene (48) [CDCl_3]



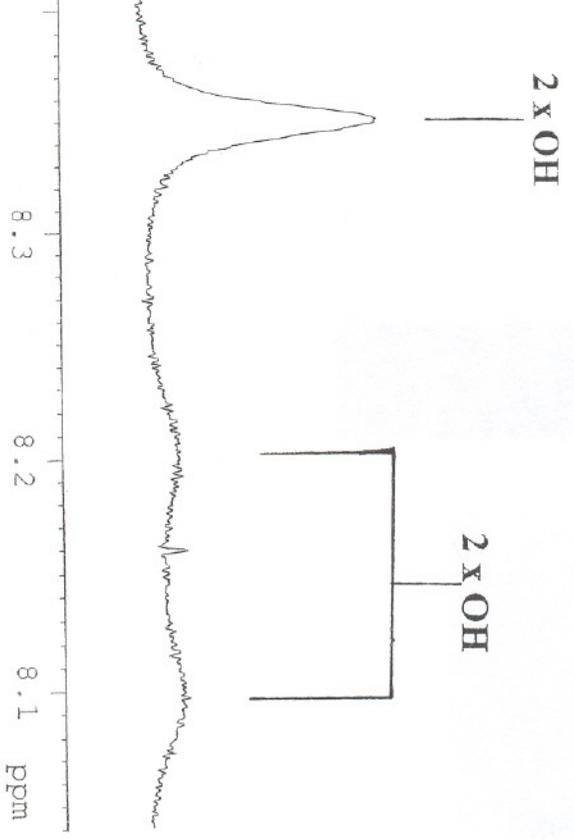
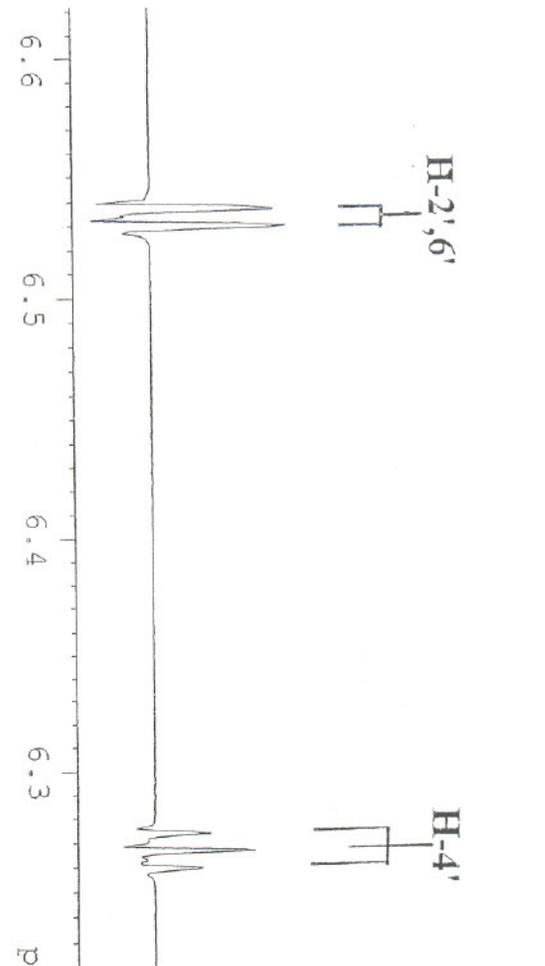
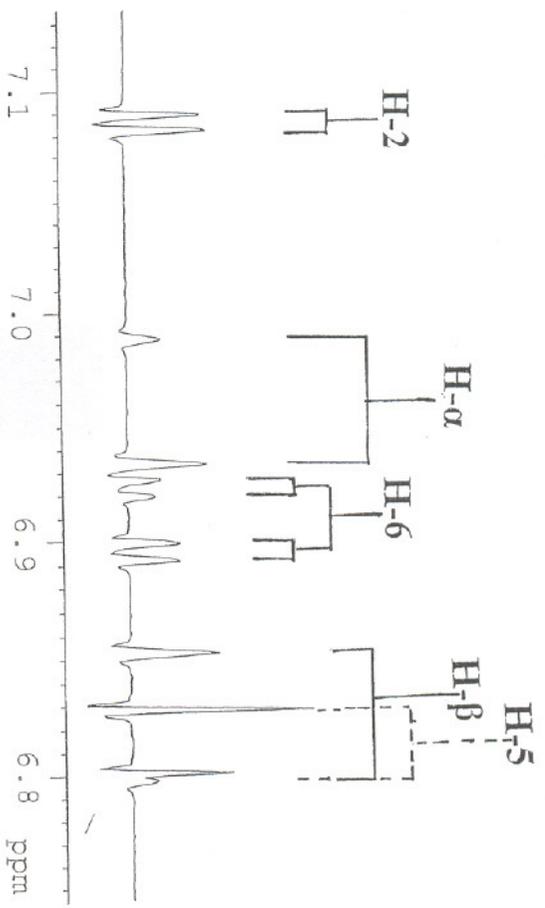


Plate 9: ^1H NMR data of *trans*-3,4,3',5'-tetraacetoxystilbene (74) [CDCl_3]

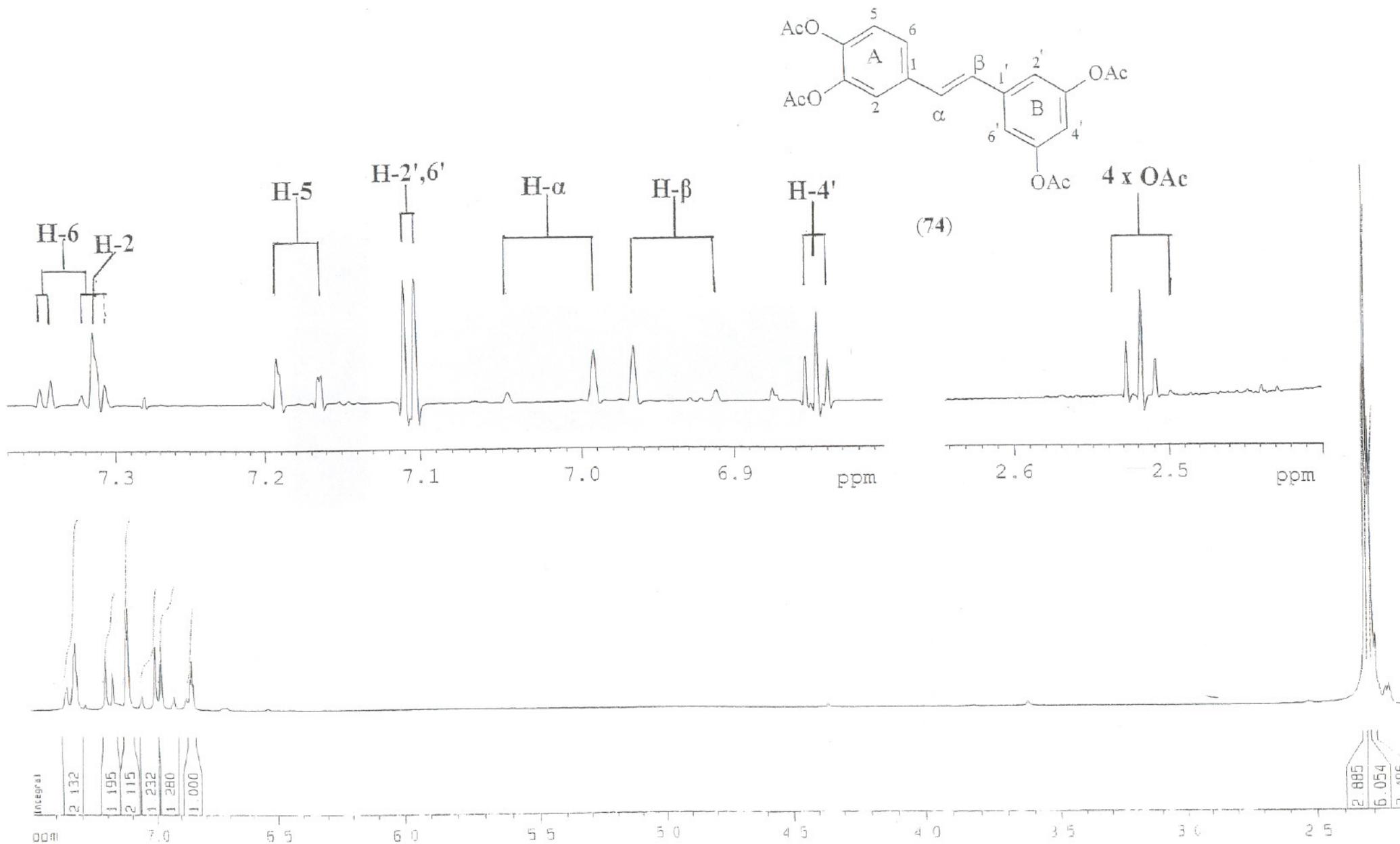


Plate 10: ¹H NMR data of *trans*-2,3',5'-triacetoxy-4-methoxystilbene (**75**) [CDCl₃]

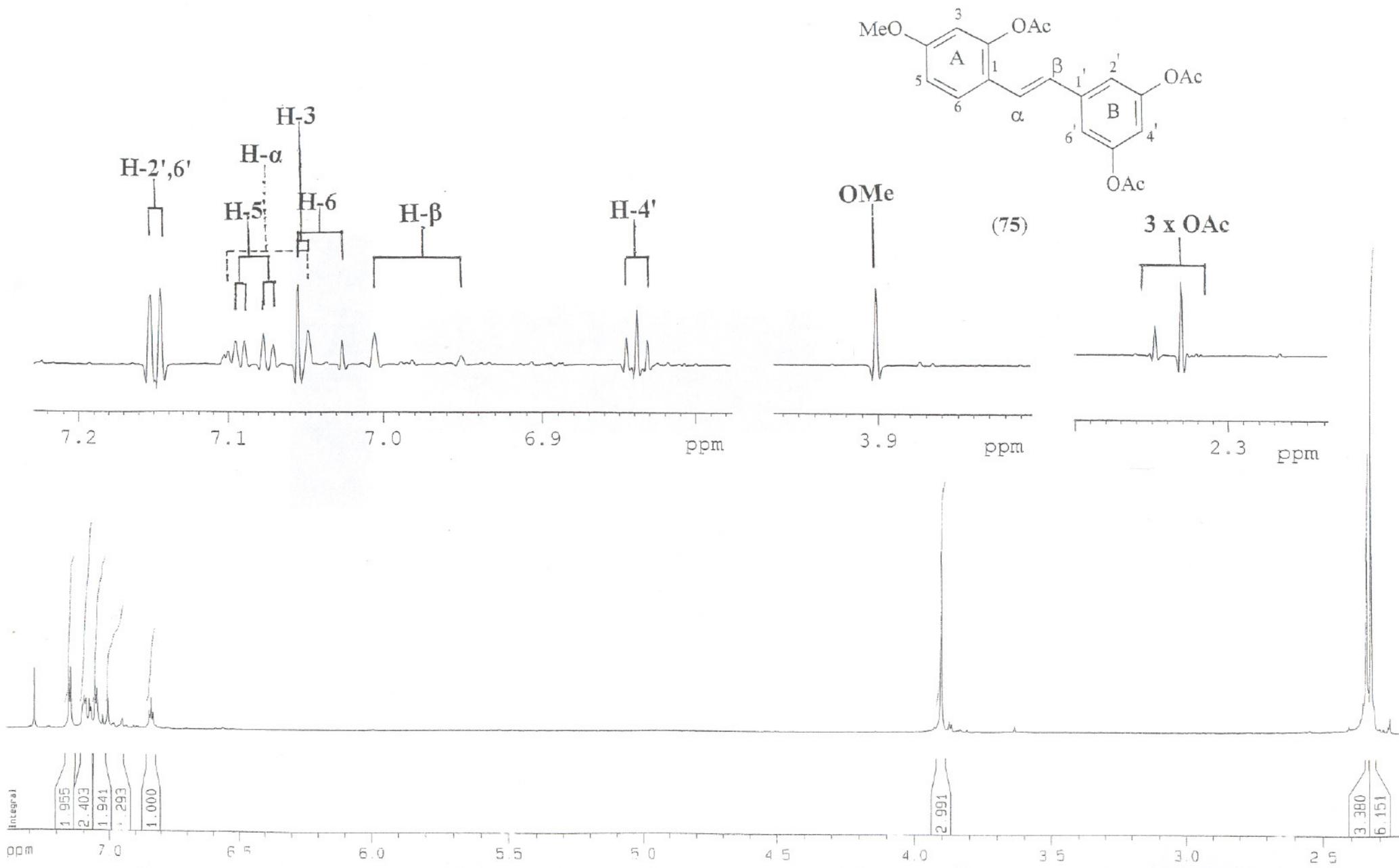
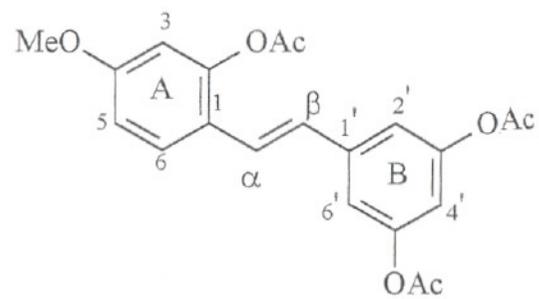
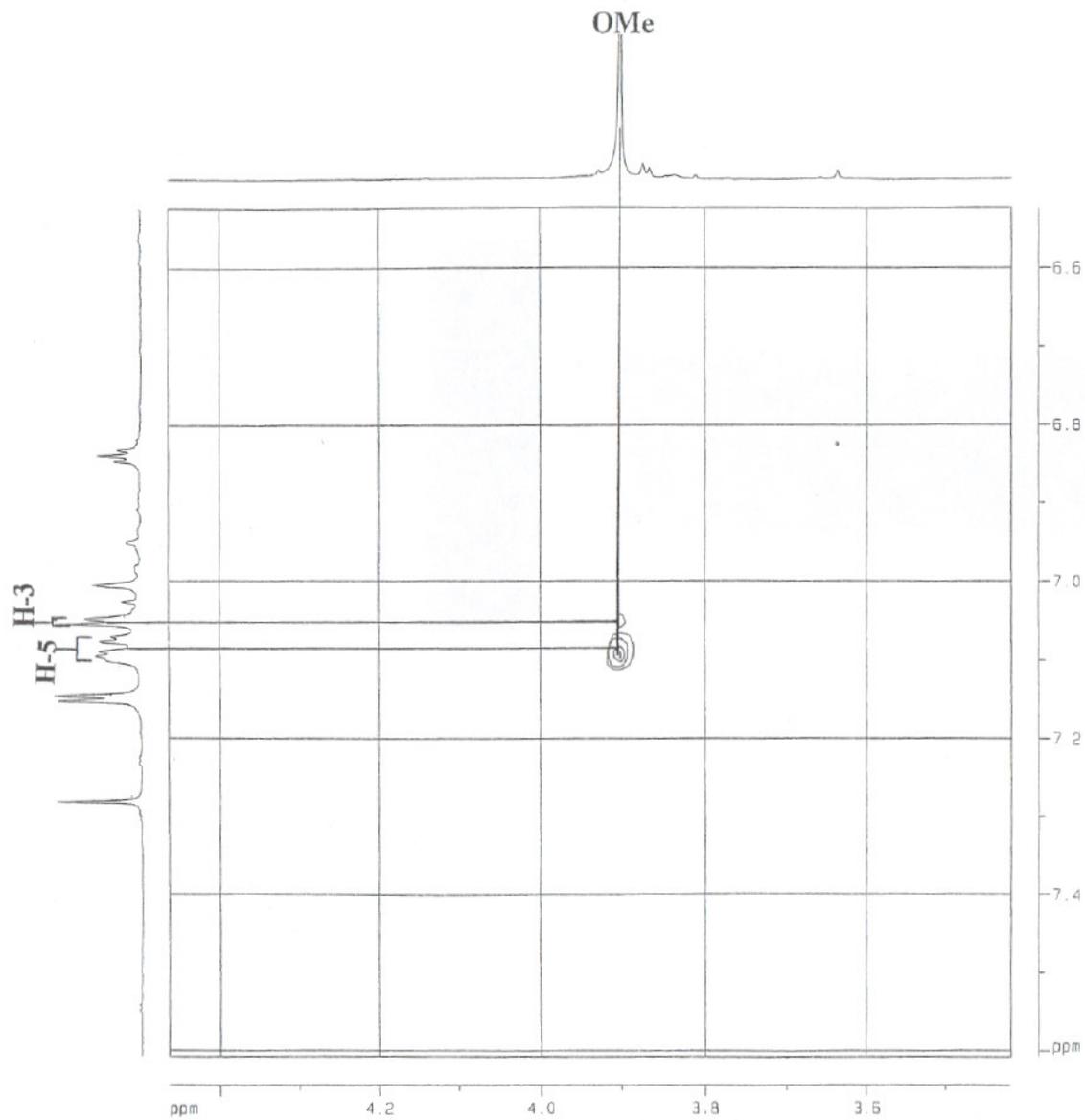


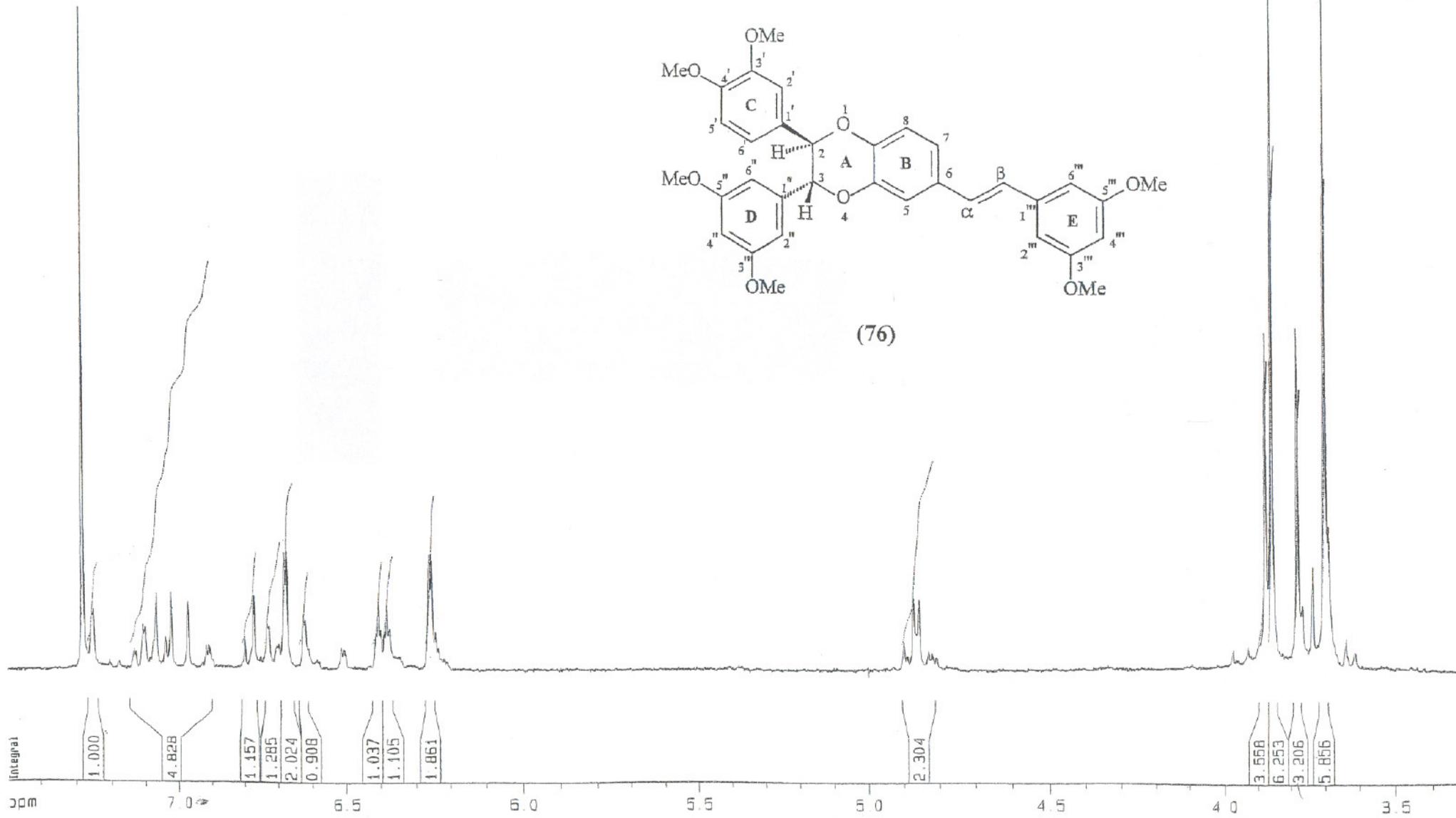
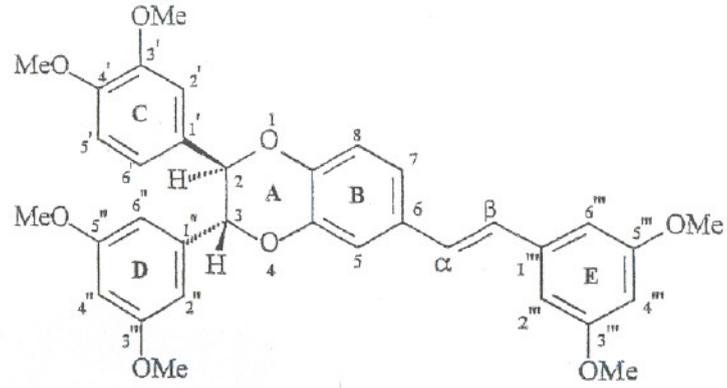
Plate 10a: NOESY of *trans*-2,3',5'-triacetoxy-4-methoxystilbene (75) [CDCl₃]



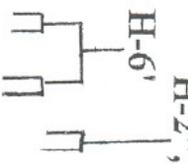
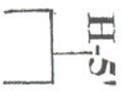
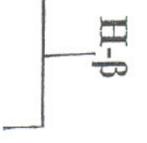
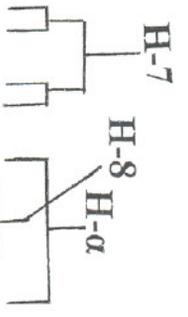
(75)



6-[2-(3,5-dimethoxyphenyl)-E-1-ethenyl]benzodioxane (76) [CDCl₃]



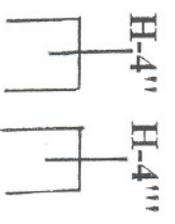
H-5



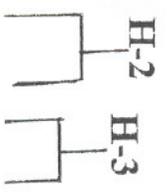
H-2''',6'''



7.2 7.1 7.0 ppm



6.40 6.35 6.30 ppm



4.9 ppm

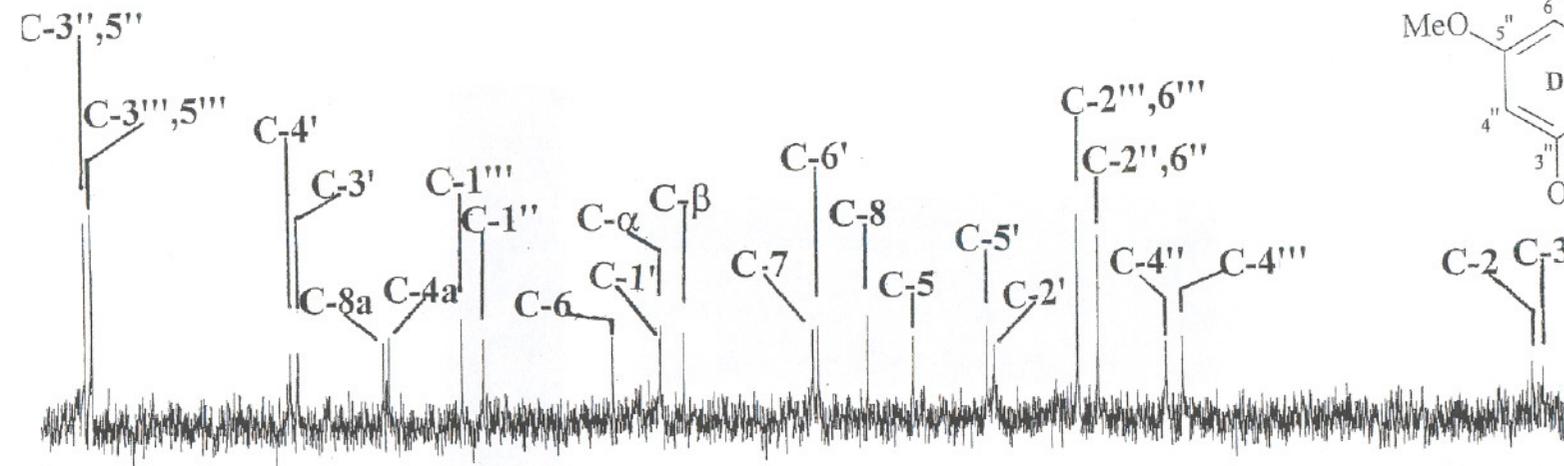
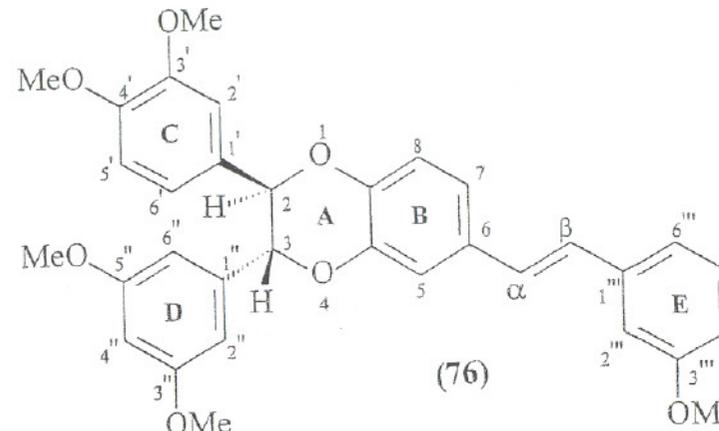


3.9 3.8 ppm

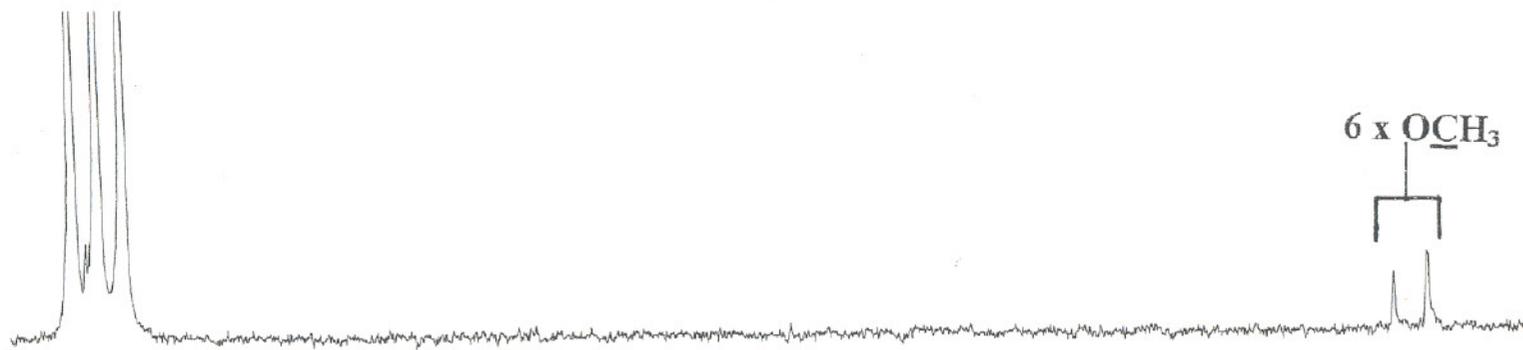
6.8 6.7 6.6 ppm

Plate 11a: ^{13}C NMR of *rel*-2,3-*trans*-2-(3,4-Dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-

6-[2-(3,5-dimethoxyphenyl)-*E*-1-ethenyl]benzodioxane (76) [CDCl_3]



ppm 150 140 130 120 110 100 90



ppm 75 70 65 60 55

Plate 11b: NOESY of *rel*-2,3-*trans*-2-(3,4-Dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-6-[2-(3,5-dimethoxyphenyl)-*E*-1-ethenyl]benzodioxane (76) [CDCl₃]

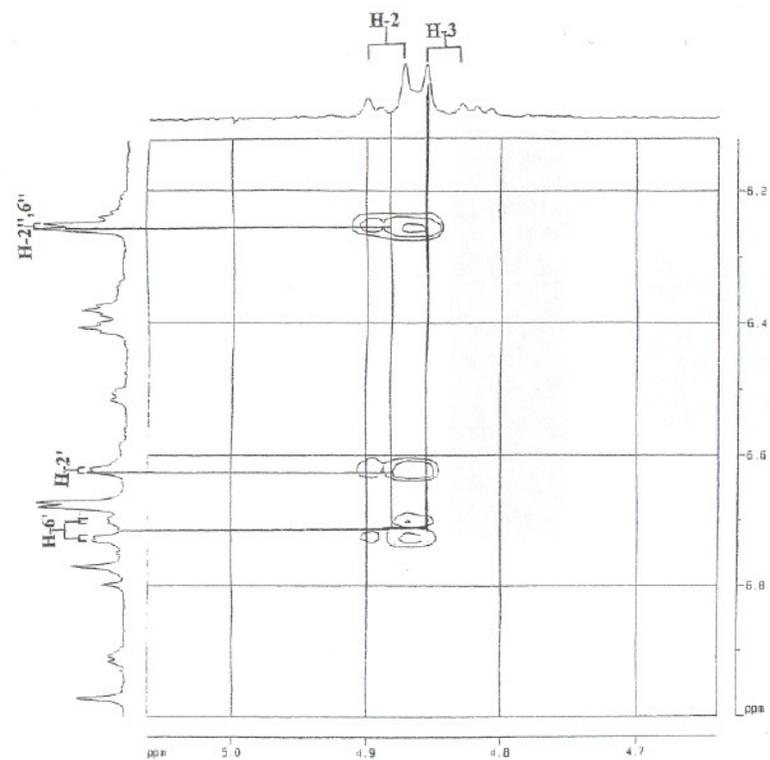


Plate 11c: NOESY of *rel*-2,3-*trans*-2-(3,4-Dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-6-[2-(3,5-dimethoxyphenyl)-*E*-1-ethenyl]benzodioxane (76) [CDCl₃]

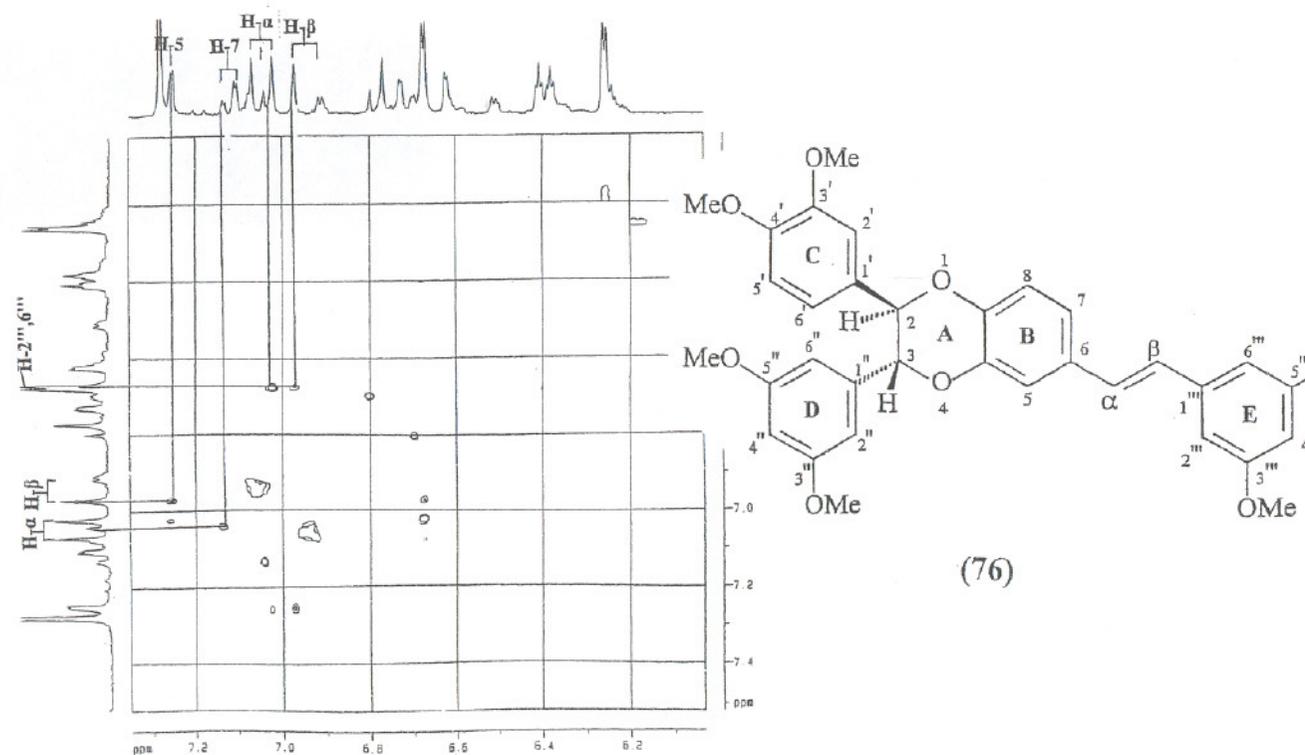
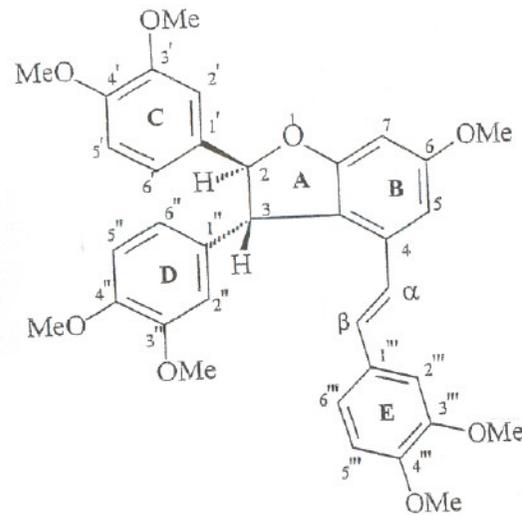
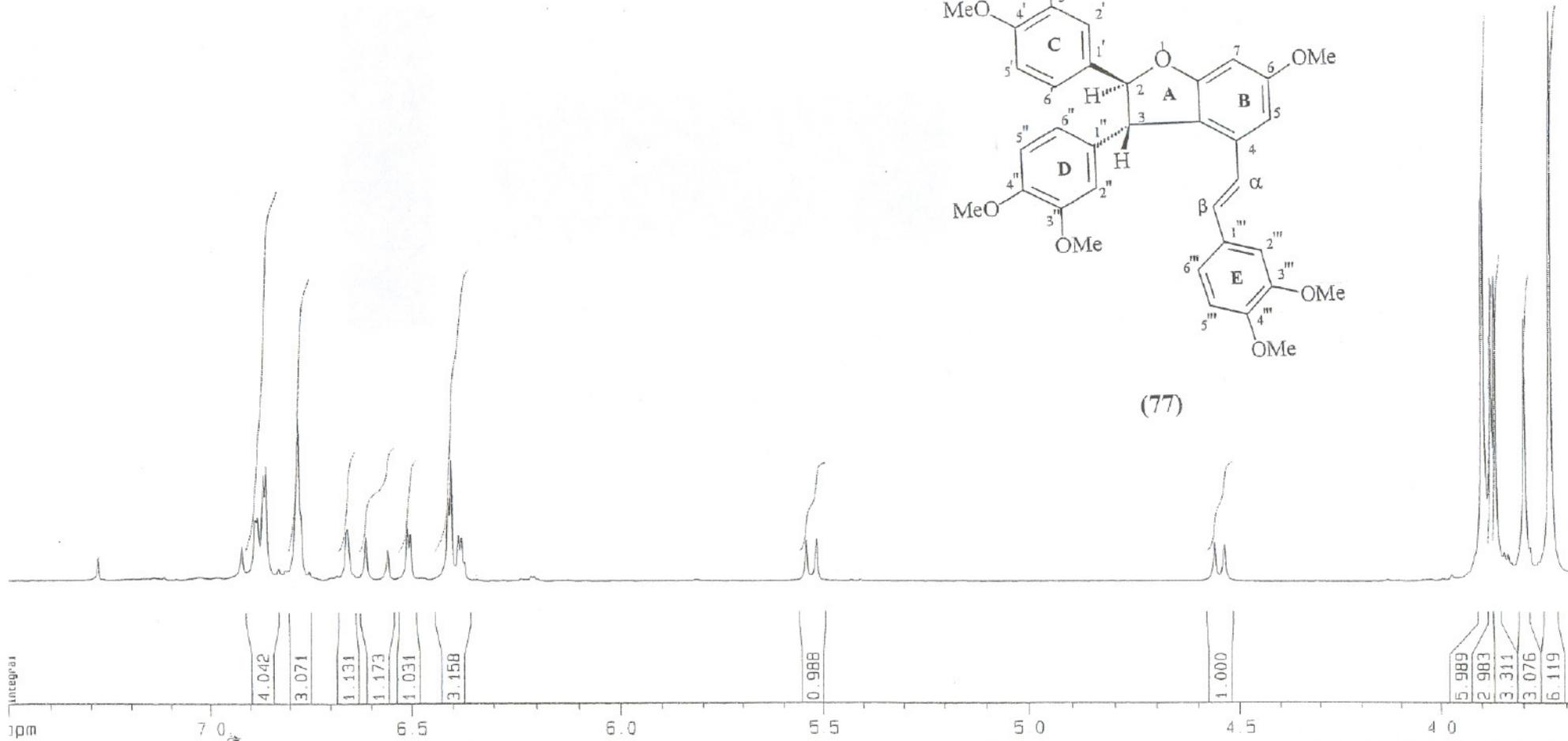
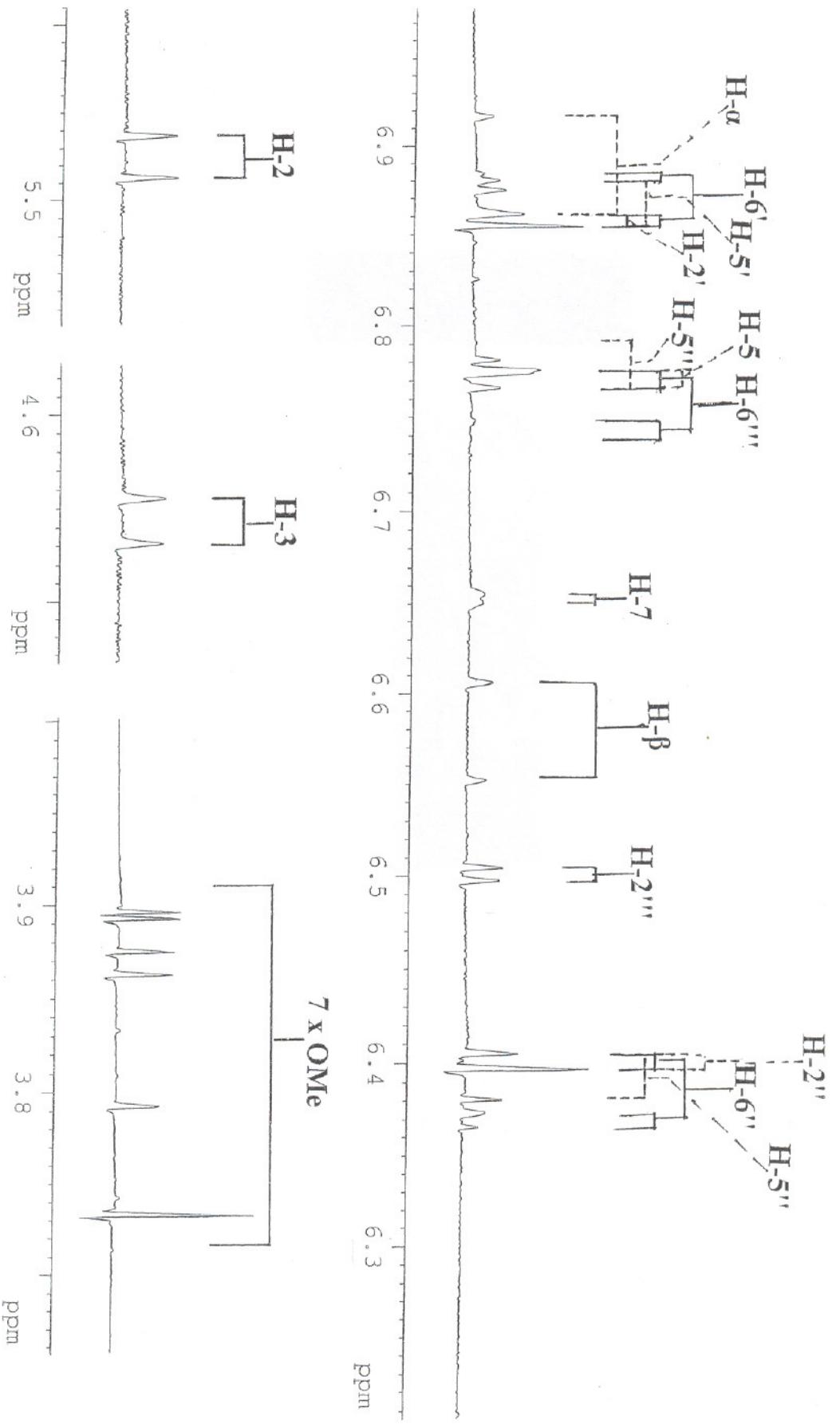


Plate 12: ^1H NMR of *rel*-2,3-*trans*-2-(3,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-4-[2-(3,4-dimethoxyphenyl)-*E*-1-ethenyl]-6-methoxy-2,3-dihydrobenzofuran (77) [CDCl_3]

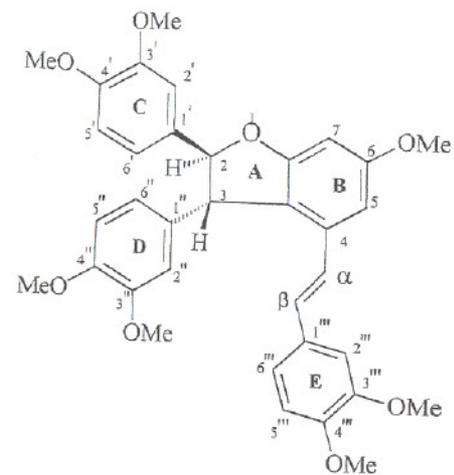
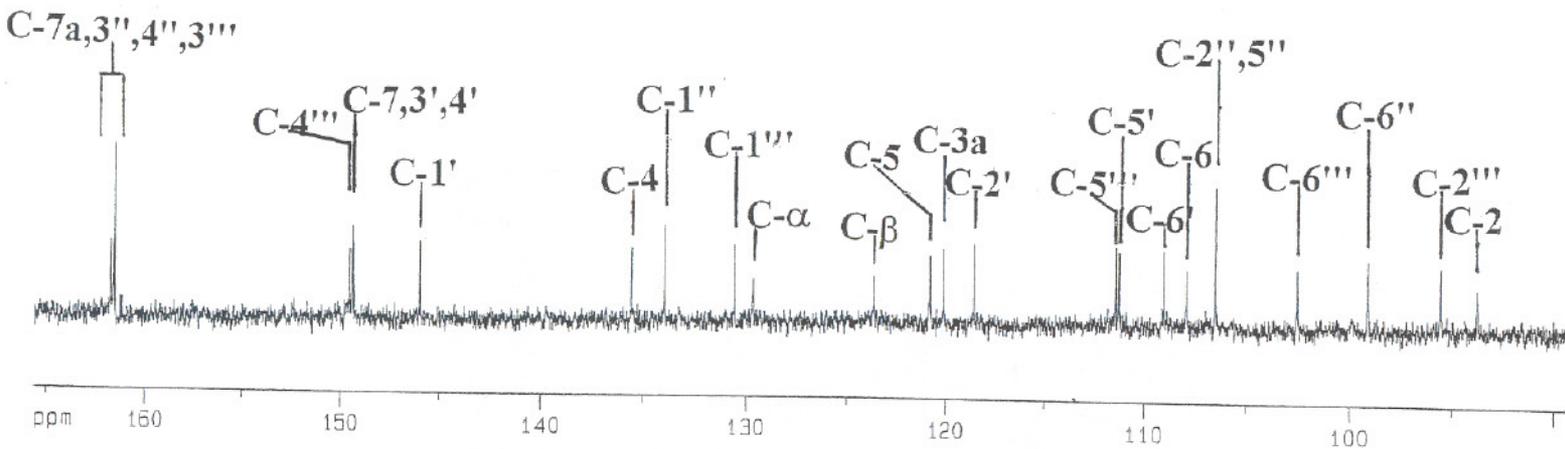


(77)





(77) [CDCl₃]



(77)

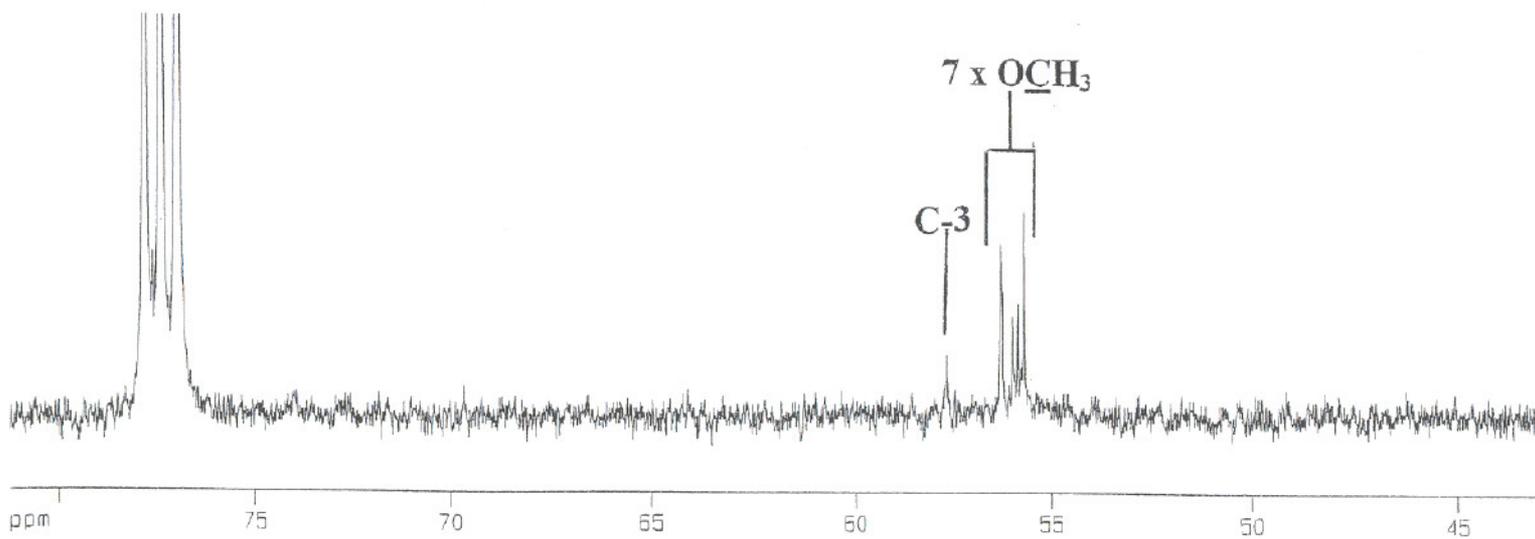


Plate 12b: NOESY of *rel*-2,3-*trans*-2-(3,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-4-[2-(3,4-dimethoxyphenyl)-*E*-1-ethenyl]-6-methoxy-2,3-dihydrobenzofuran (77) [CDCl₃]

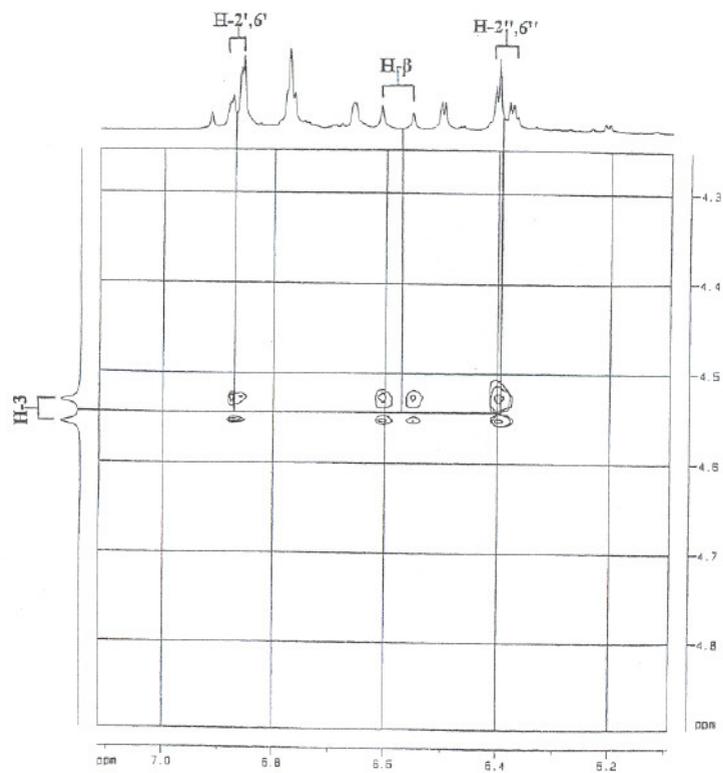
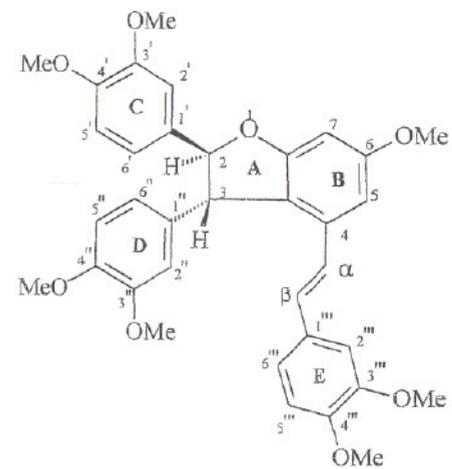
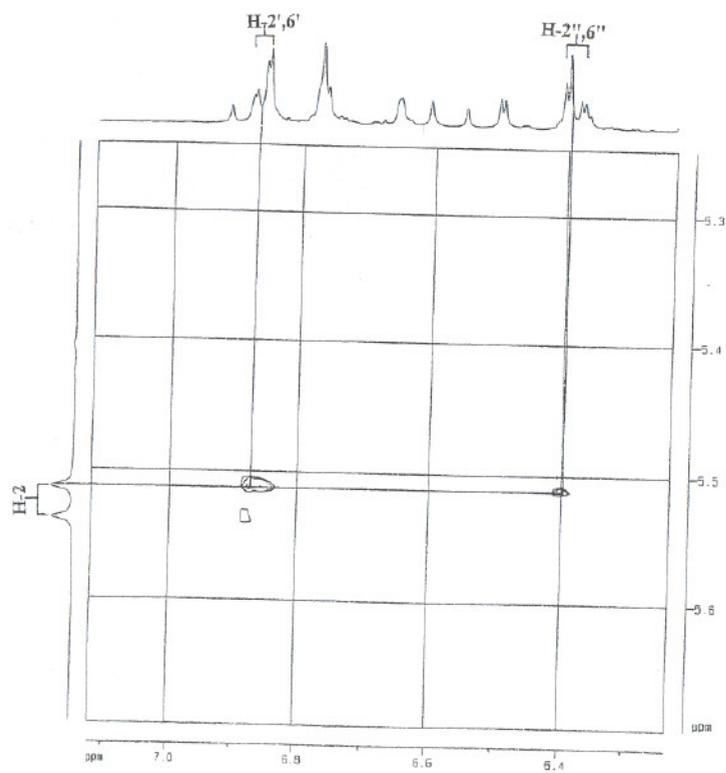
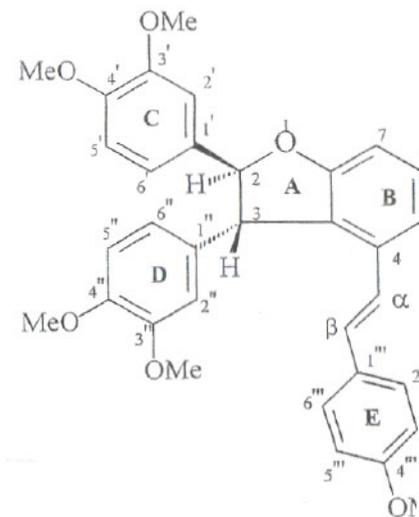
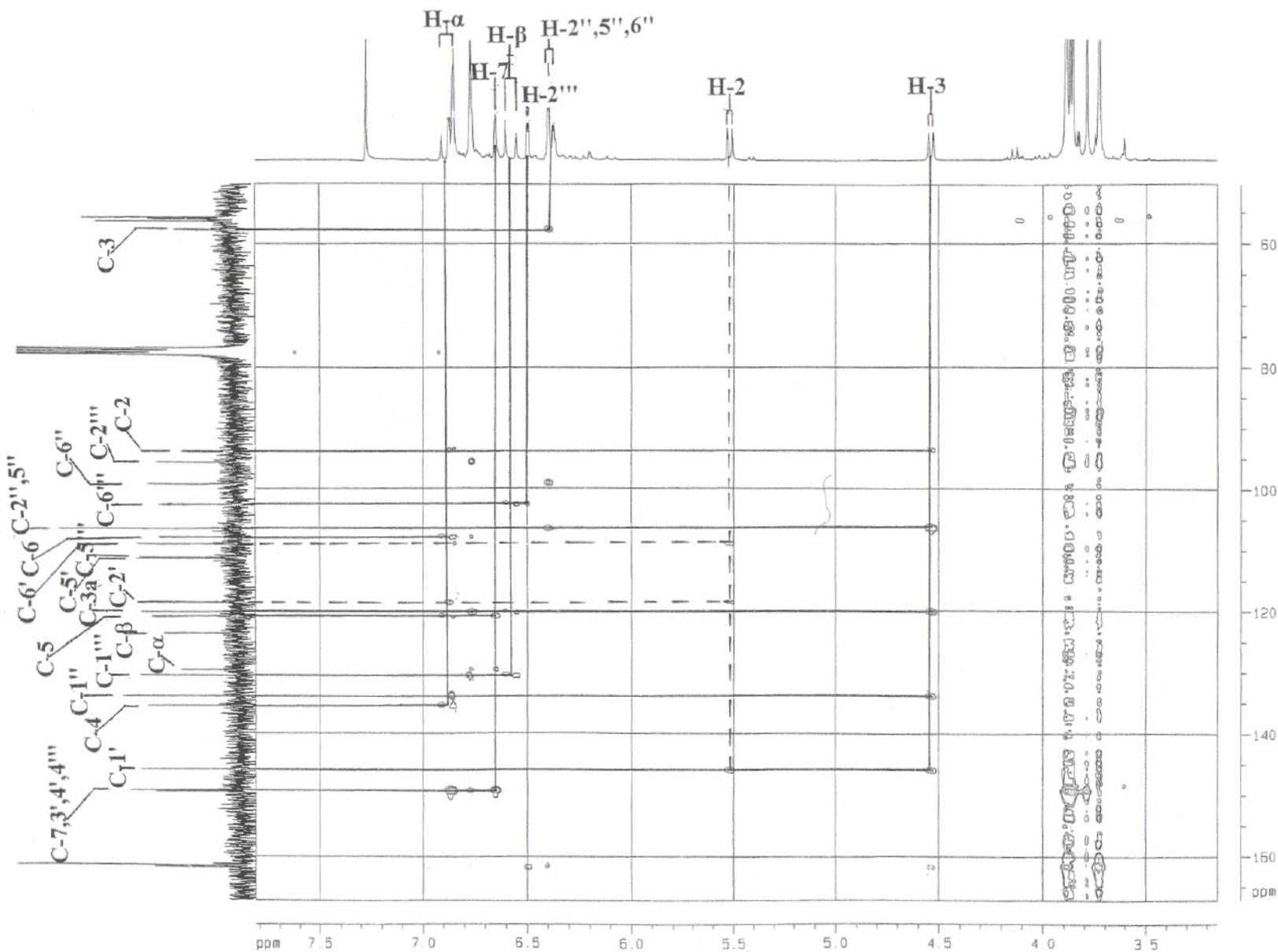


Plate 12c: NOESY of *rel*-2,3-*trans*-2-(3,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-4-[2-(3,4-dimethoxyphenyl)-*E*-1-ethenyl]-6-methoxy-2,3-dihydrobenzofuran (77) [CDCl₃]



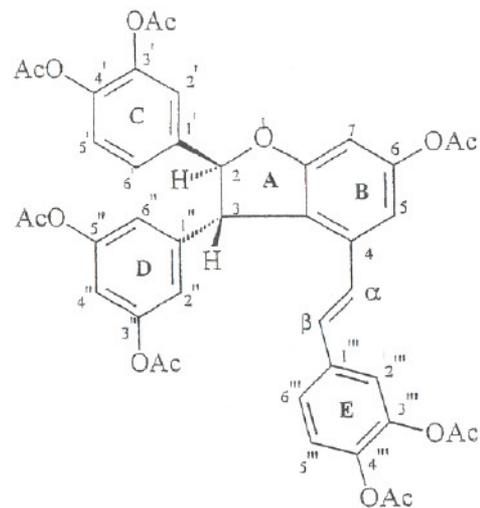
(77)

Plate 12d: HMBC of *rel*-2,3-*trans*-2-(3,4-Dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-4-[2-(3,4- dimethoxyphenyl)]-*E*-1-ethenyl]-6-methoxy-2,3-dihydrobenzofuran (77) [CDCl₃]

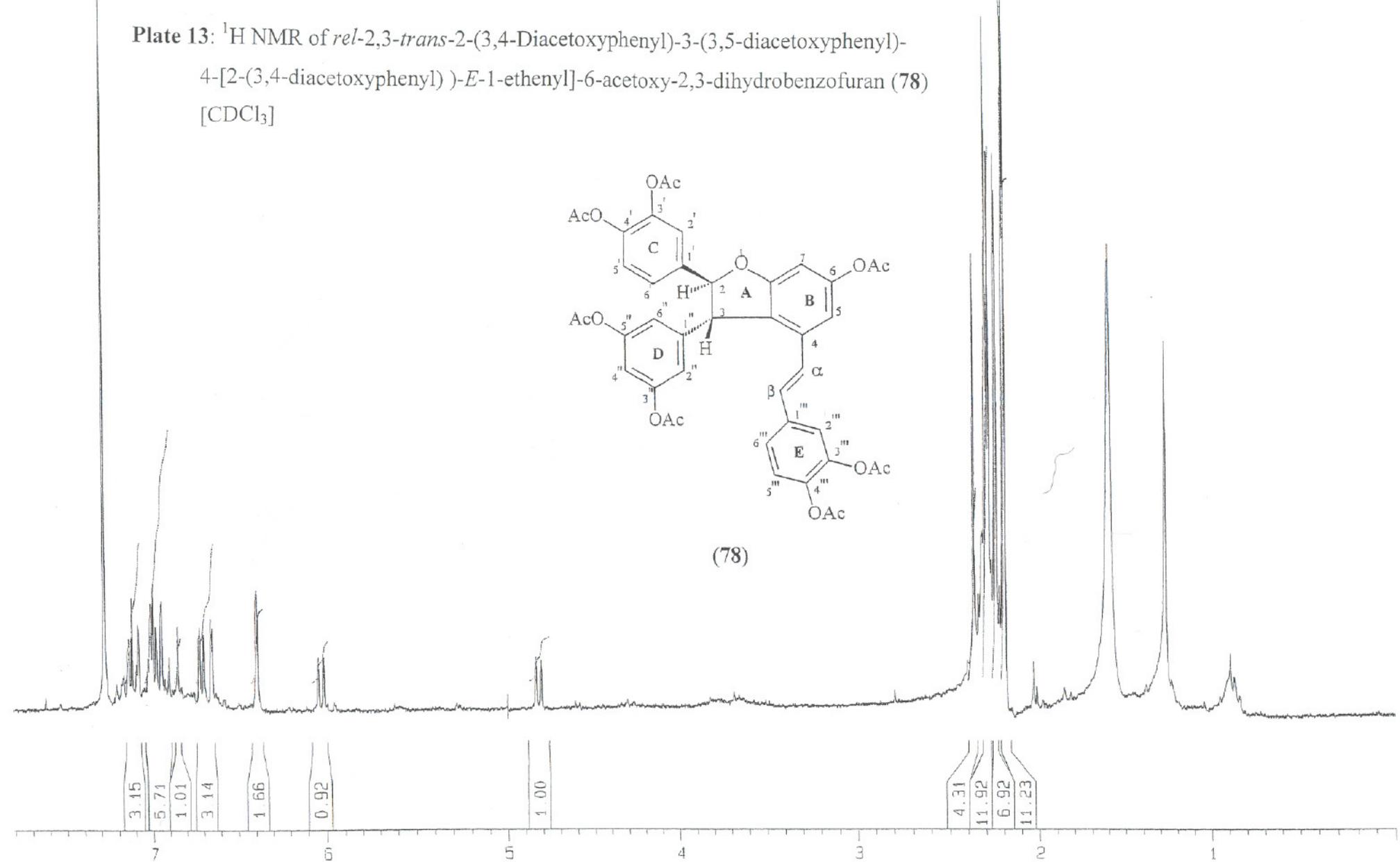


(77)

Plate 13: ^1H NMR of *rel*-2,3-*trans*-2-(3,4-Diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-4-[2-(3,4-diacetoxyphenyl)-*E*-1-ethenyl]-6-acetoxy-2,3-dihydrobenzofuran (78)
[CDCl_3]



(78)



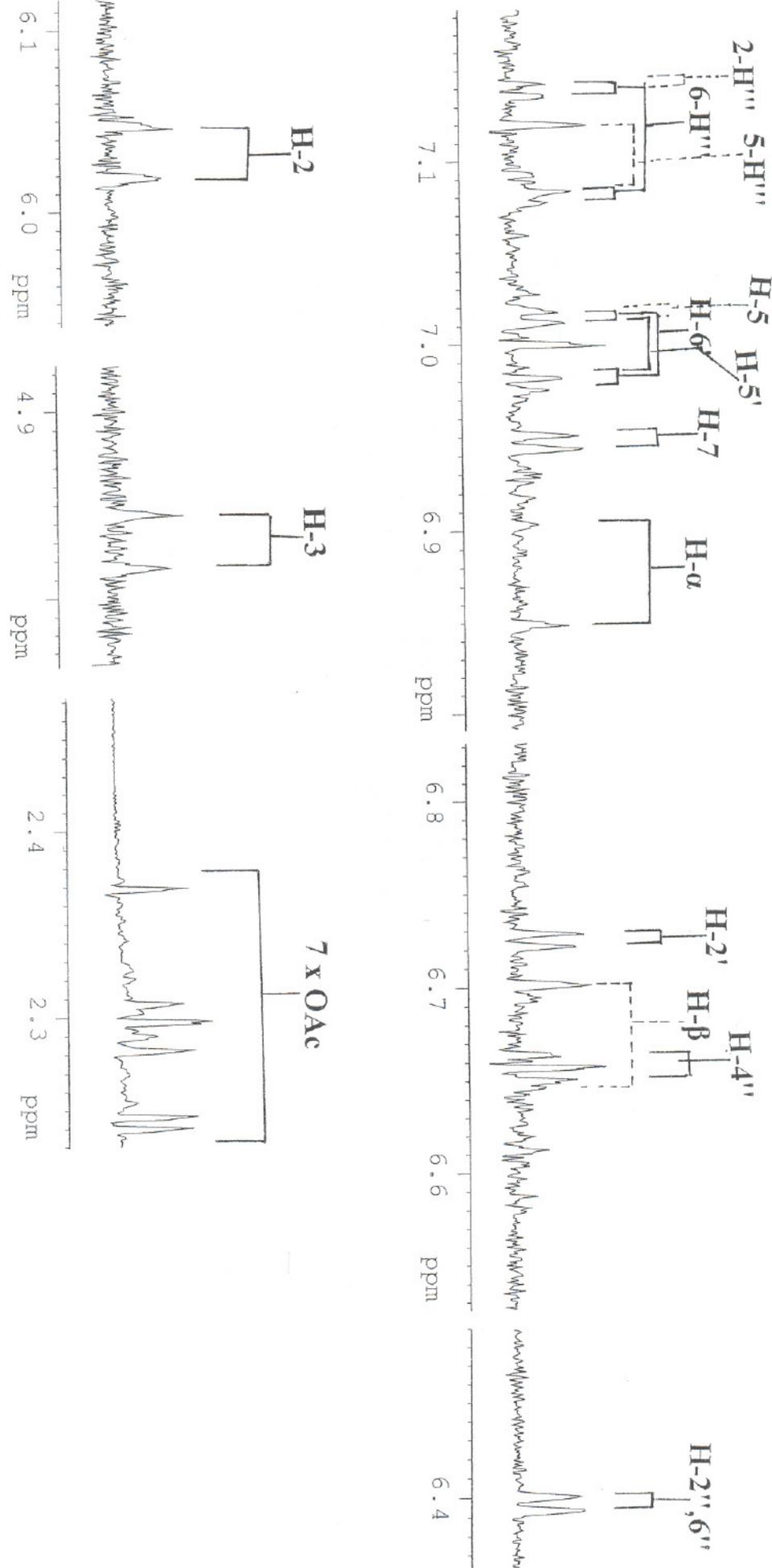
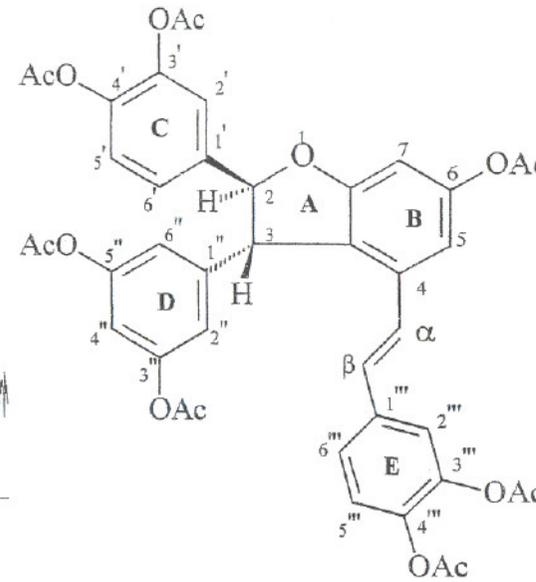
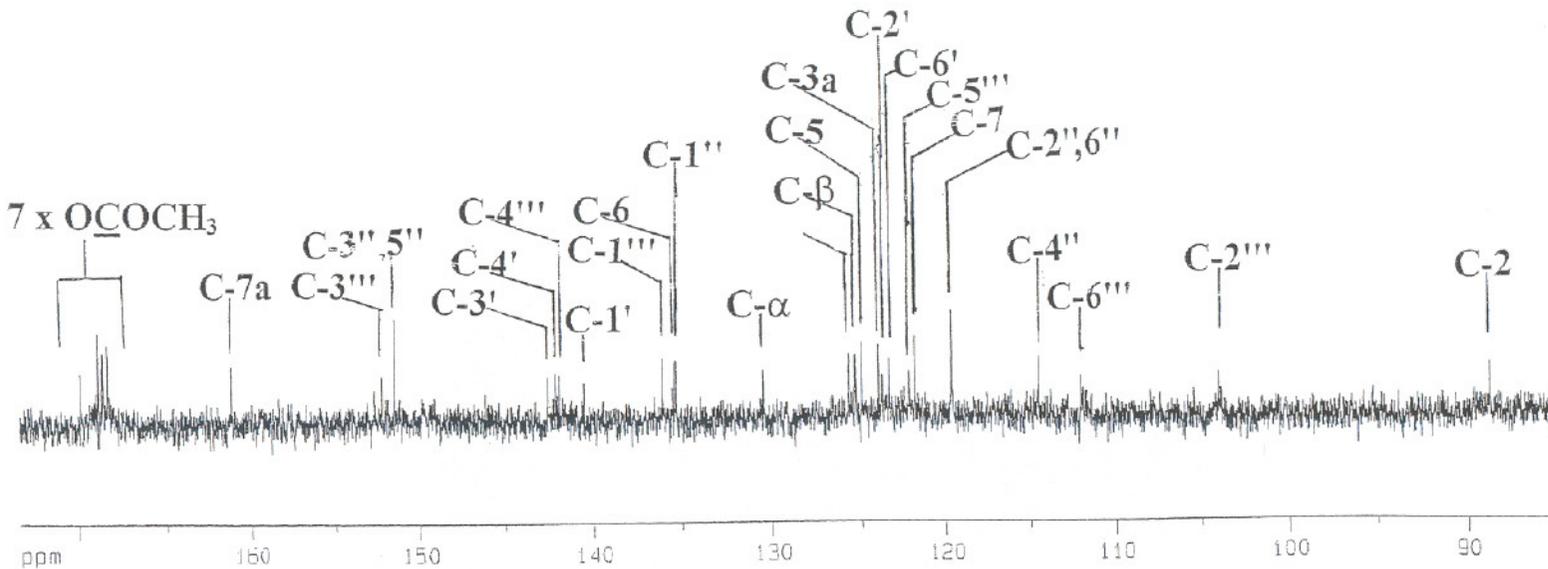


Plate 13a: ^{13}C NMR of *rel*-2,3-*trans*-2-(3,4-Diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-4-[2-(3,4-diacetoxyphenyl)-*E*-1-ethenyl]-6-acetoxy-2,3-dihydrobenzofuran (78)
 [CDCl₃]



(78)

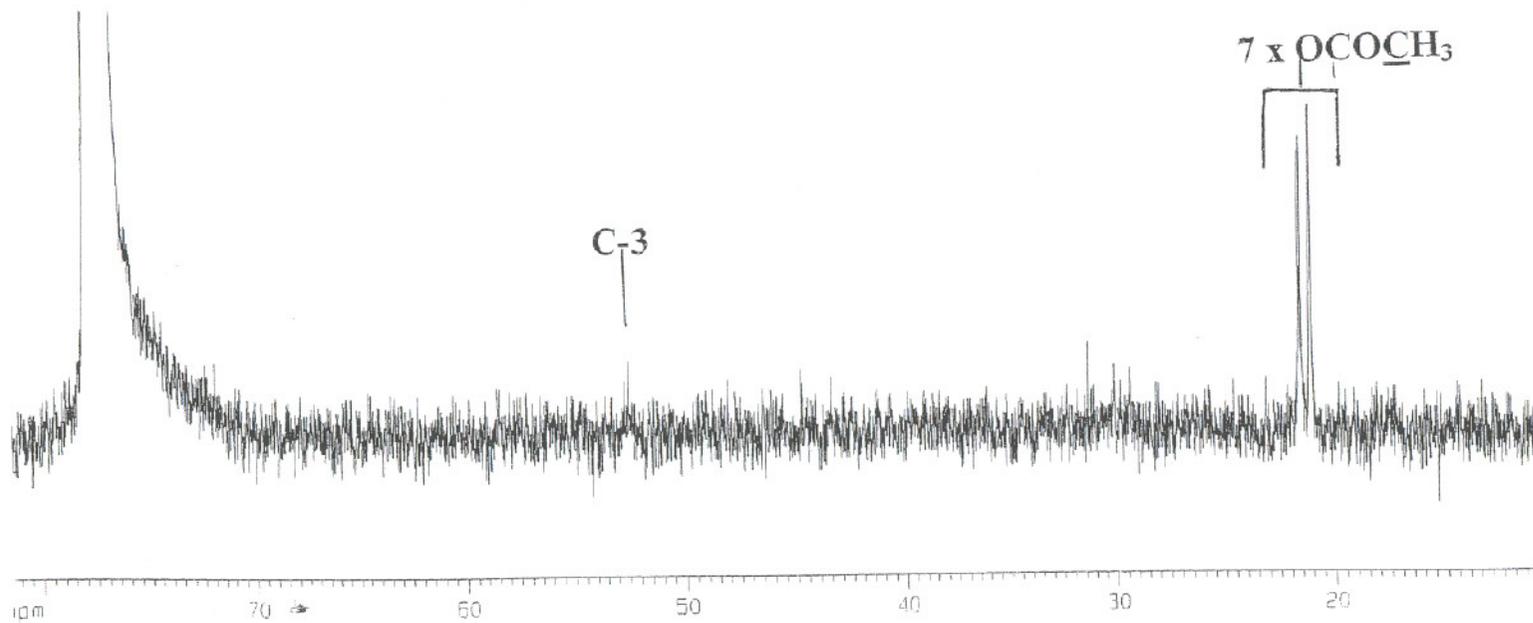


Plate 13b: NOESY *rel*-2,3-*trans*-2-(3,4-Diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-

4-[2-(3,4-diacetoxyphenyl)-*E*-1-ethenyl]-6-acetoxy-2,3-dihydrobenzofuran (78)

[CDCl₃]

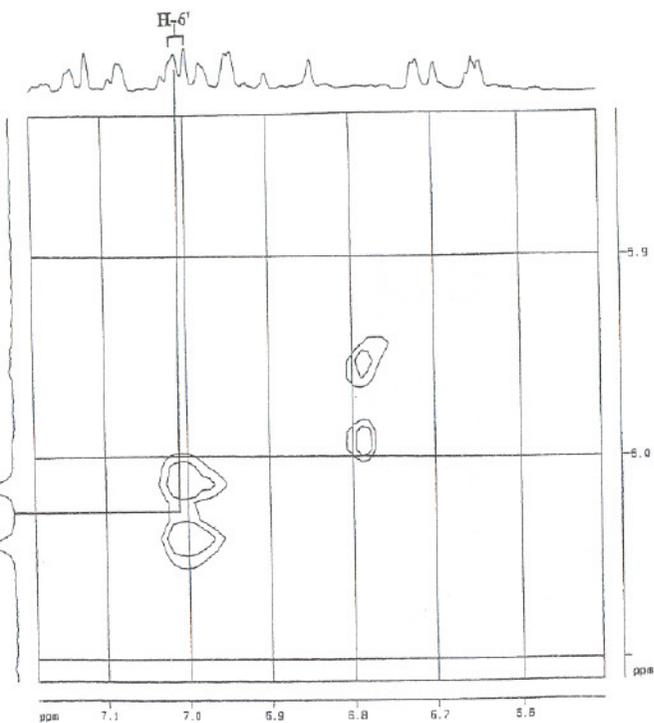
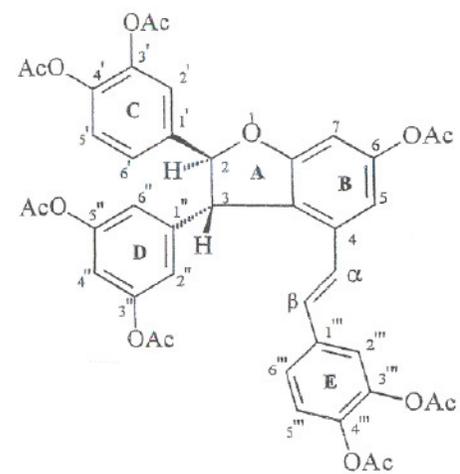
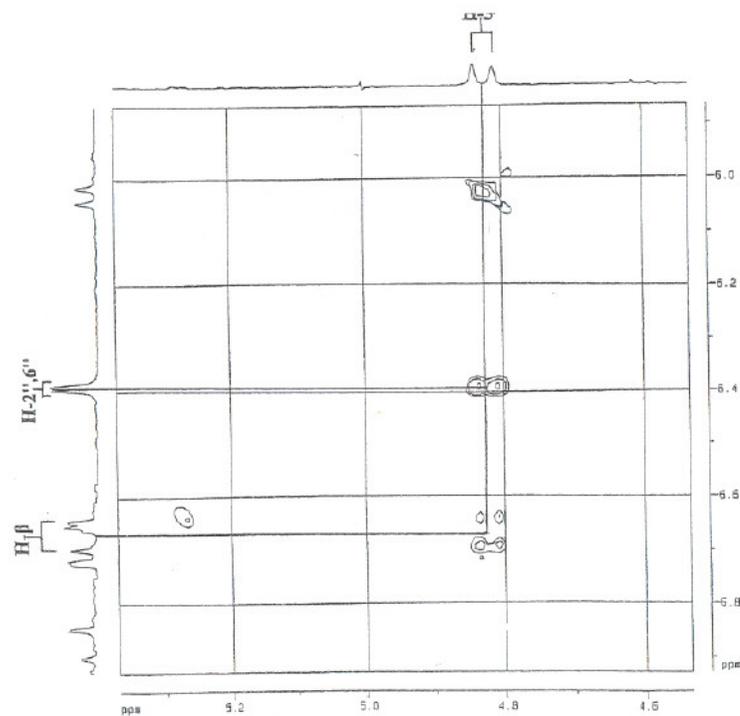


Plate 13c: NOESY of *rel*-2,3-*trans*-2-(3,4-Diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-

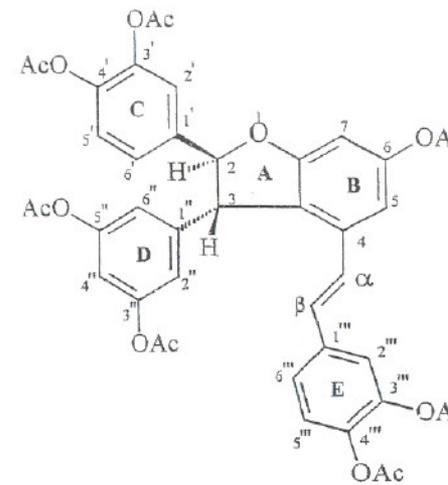
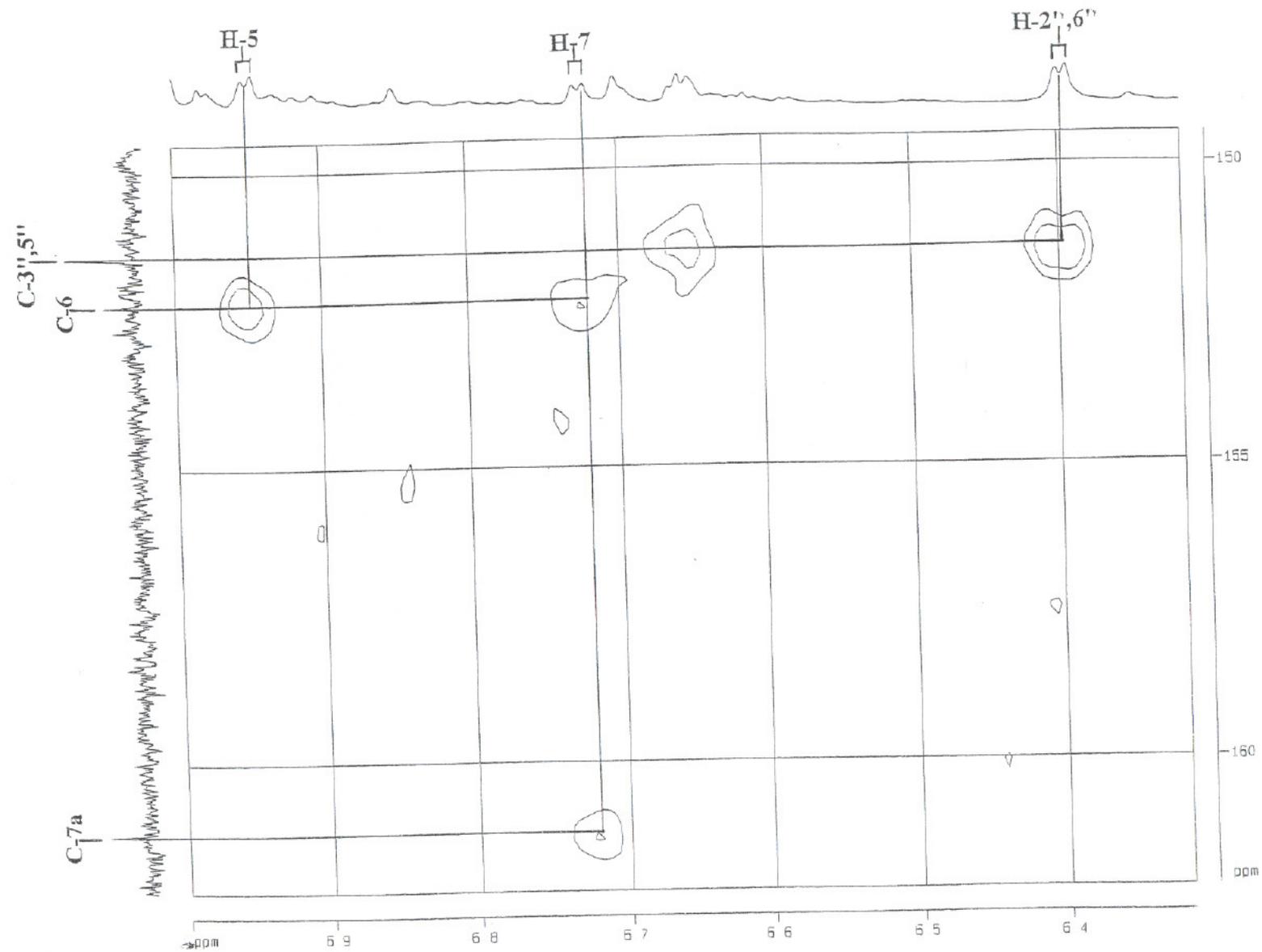
4-[2-(3,4-diacetoxyphenyl)-*E*-1-ethenyl]-6-acetoxy-2,3-dihydrobenzofuran (78)

[CDCl₃]



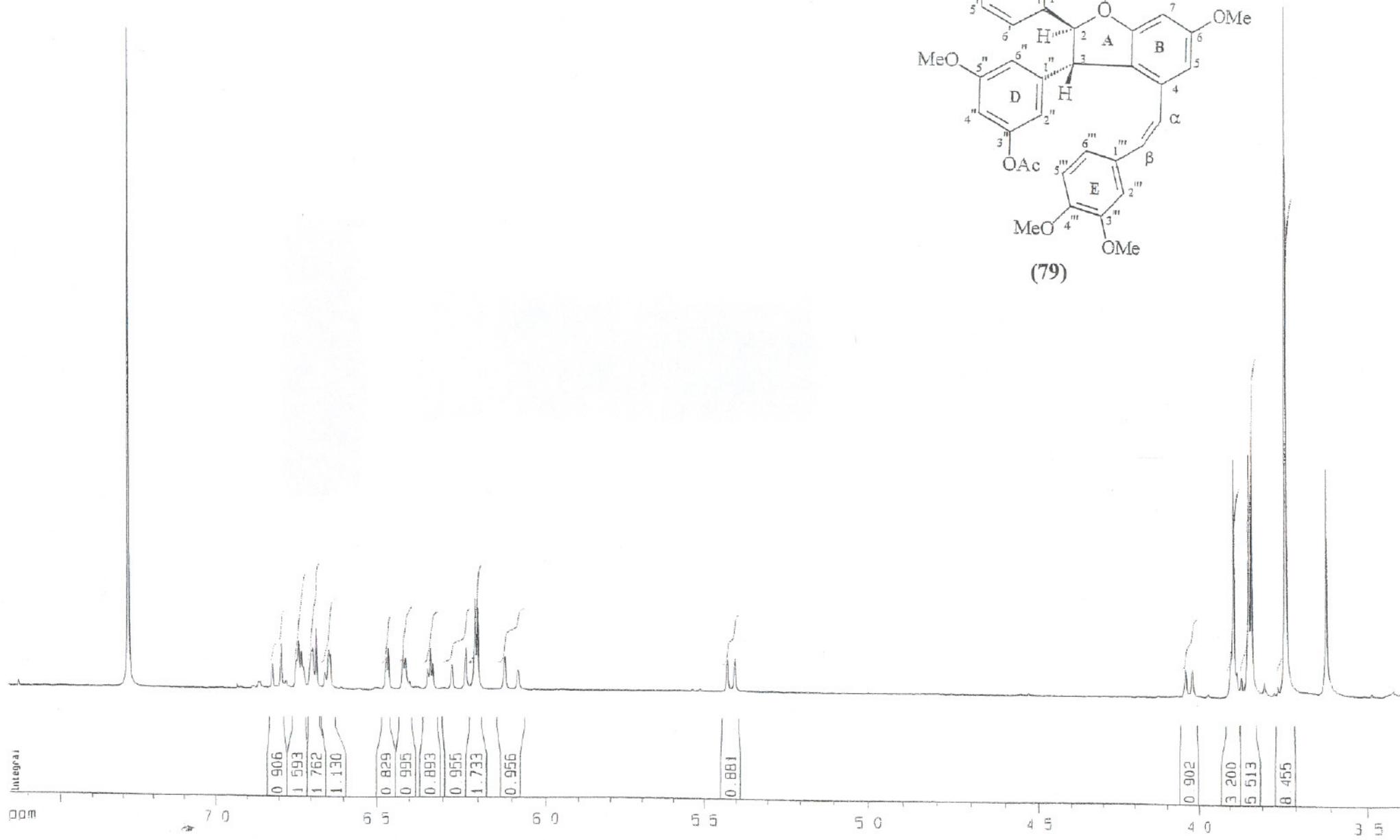
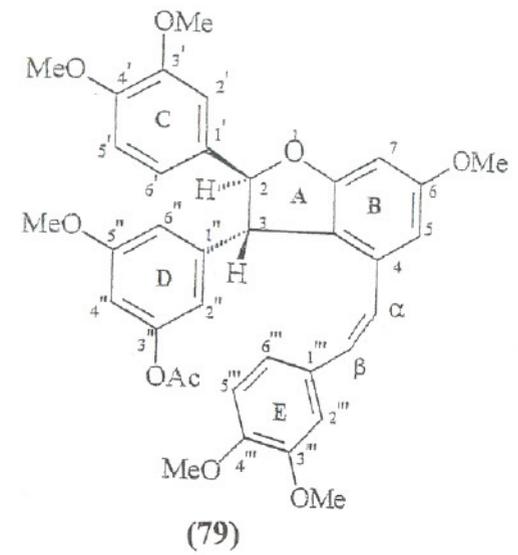
(78)

Plate 13d: HMBC of *rel*-2,3-*trans*-2-(3,4-Diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-4-[2-(3,4-diacetoxyphenyl)-*E*-1-ethenyl]-6-acetoxy-2,3-dihydrobenzofuran (78)
 [CDCl₃]



(78)

Plate 14: ^1H NMR of *rel*-2,3-*trans*-2-(3,4-dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-4-[2-(3,5-dimethoxyphenyl)-*Z*-1-ethenyl]-6-methoxy-2,3-dihydrobenzofuran (79) [CDCl_3]



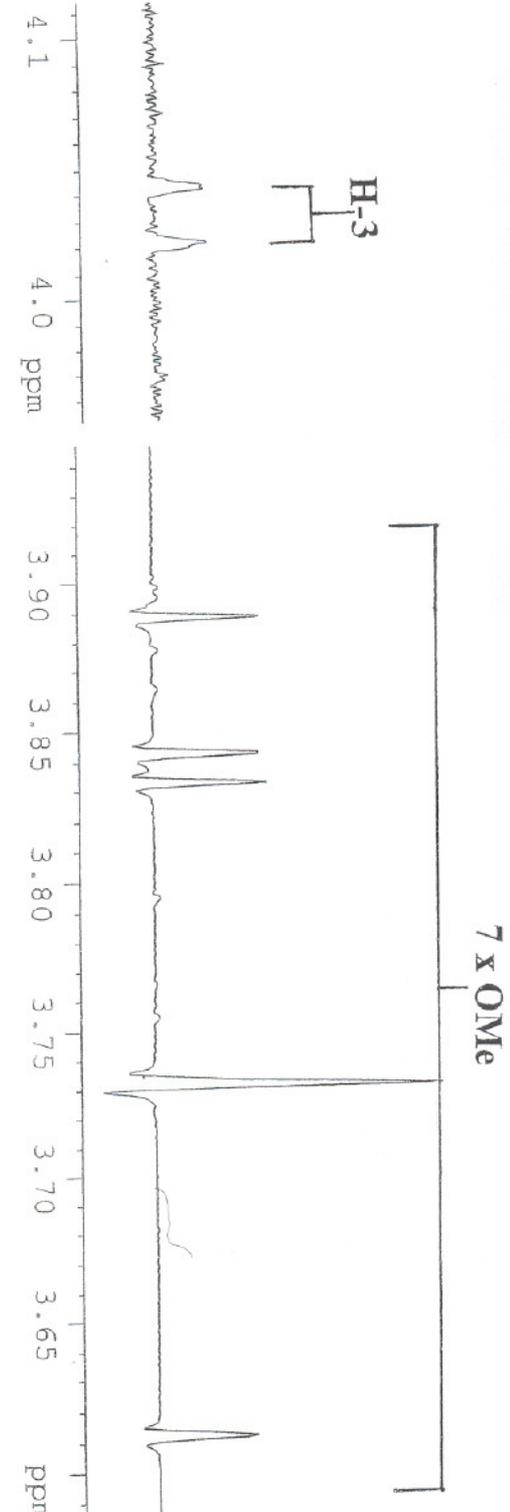
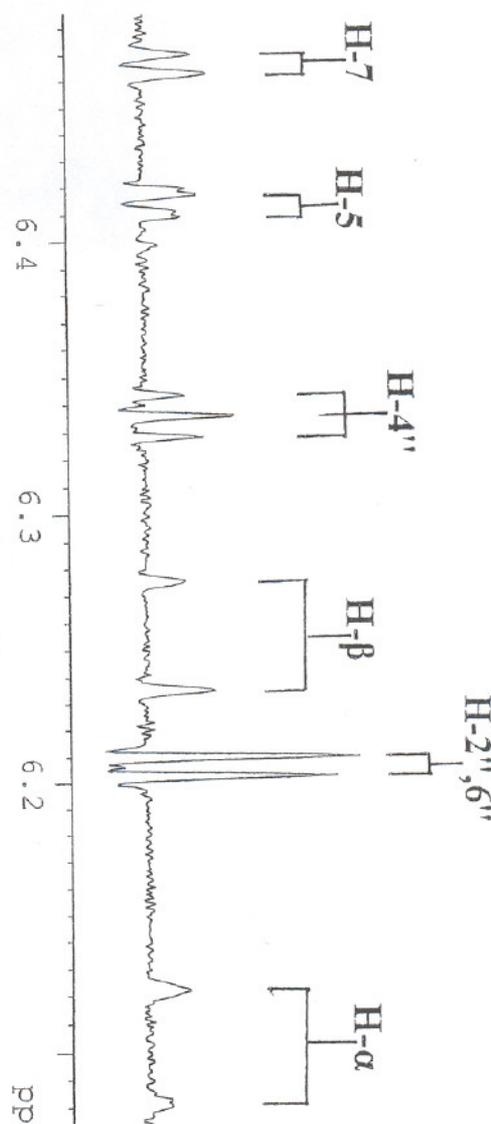
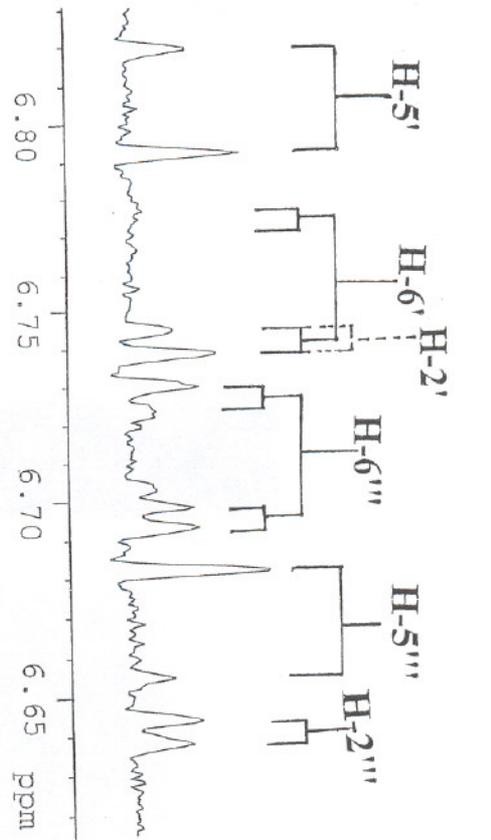
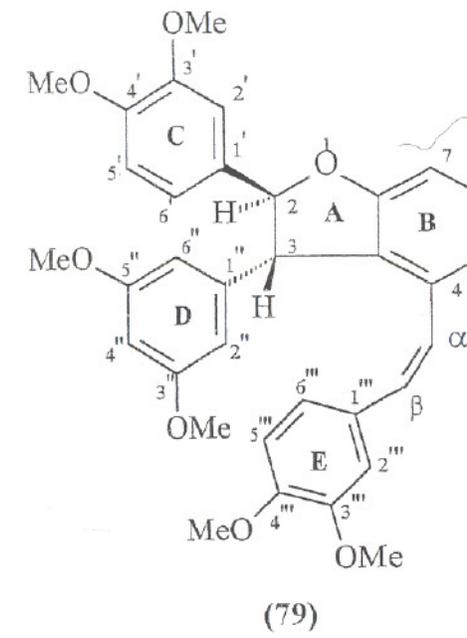
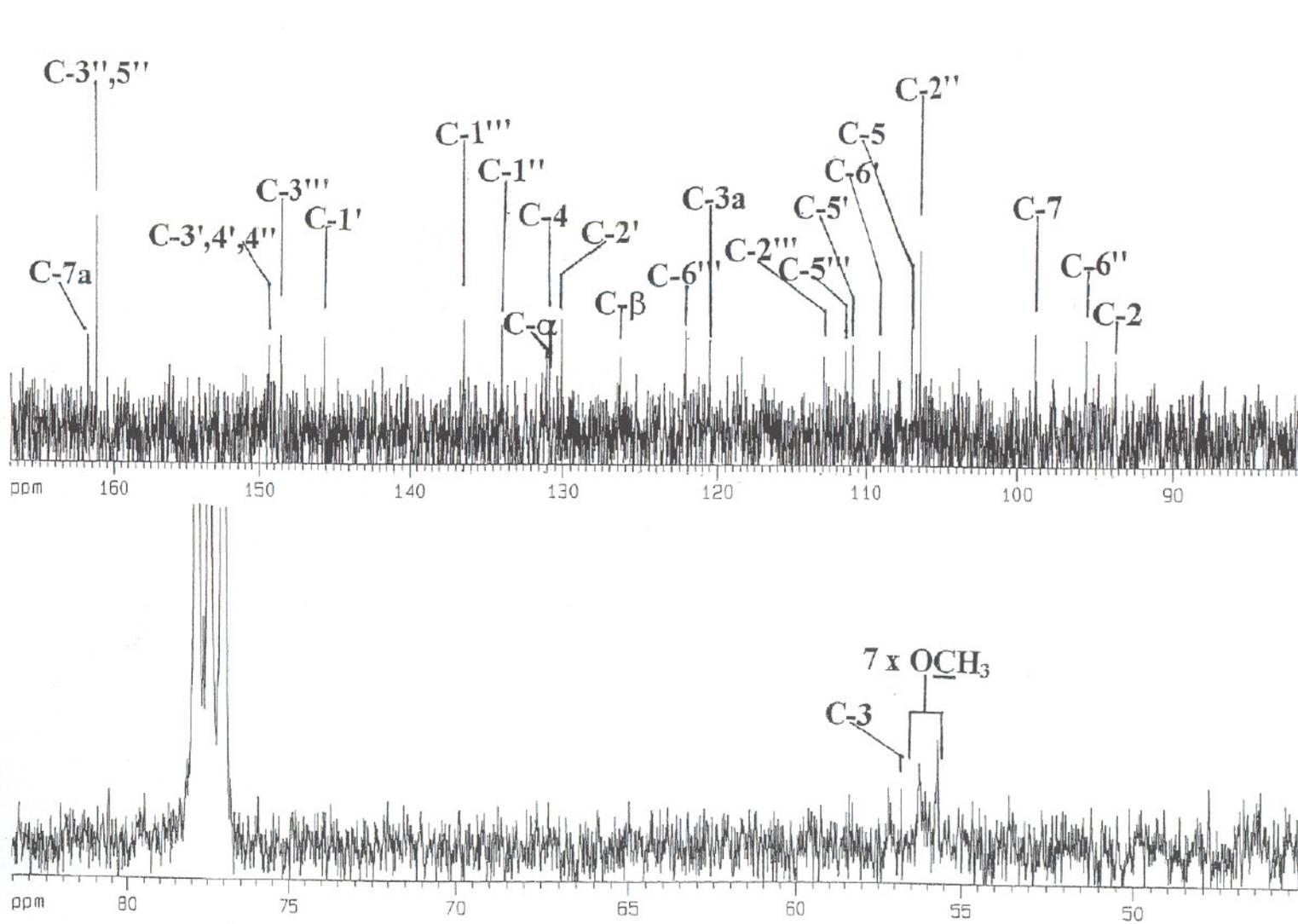


Plate 14a: ^{13}C NMR of *rel*-2,3-*trans*-2-(3,4-dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-4-[2-(3,5-dimethoxyphenyl)-*Z*-1-ethenyl]-6-methoxy-2,3-dihydrobenzofuran (79) [CDCl_3]



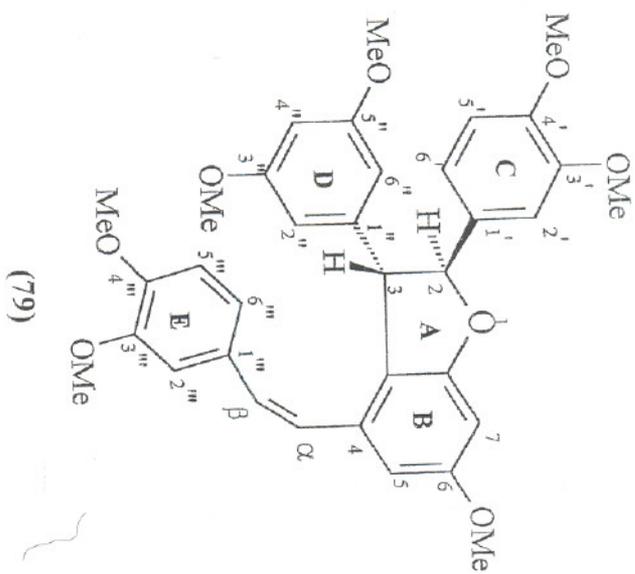
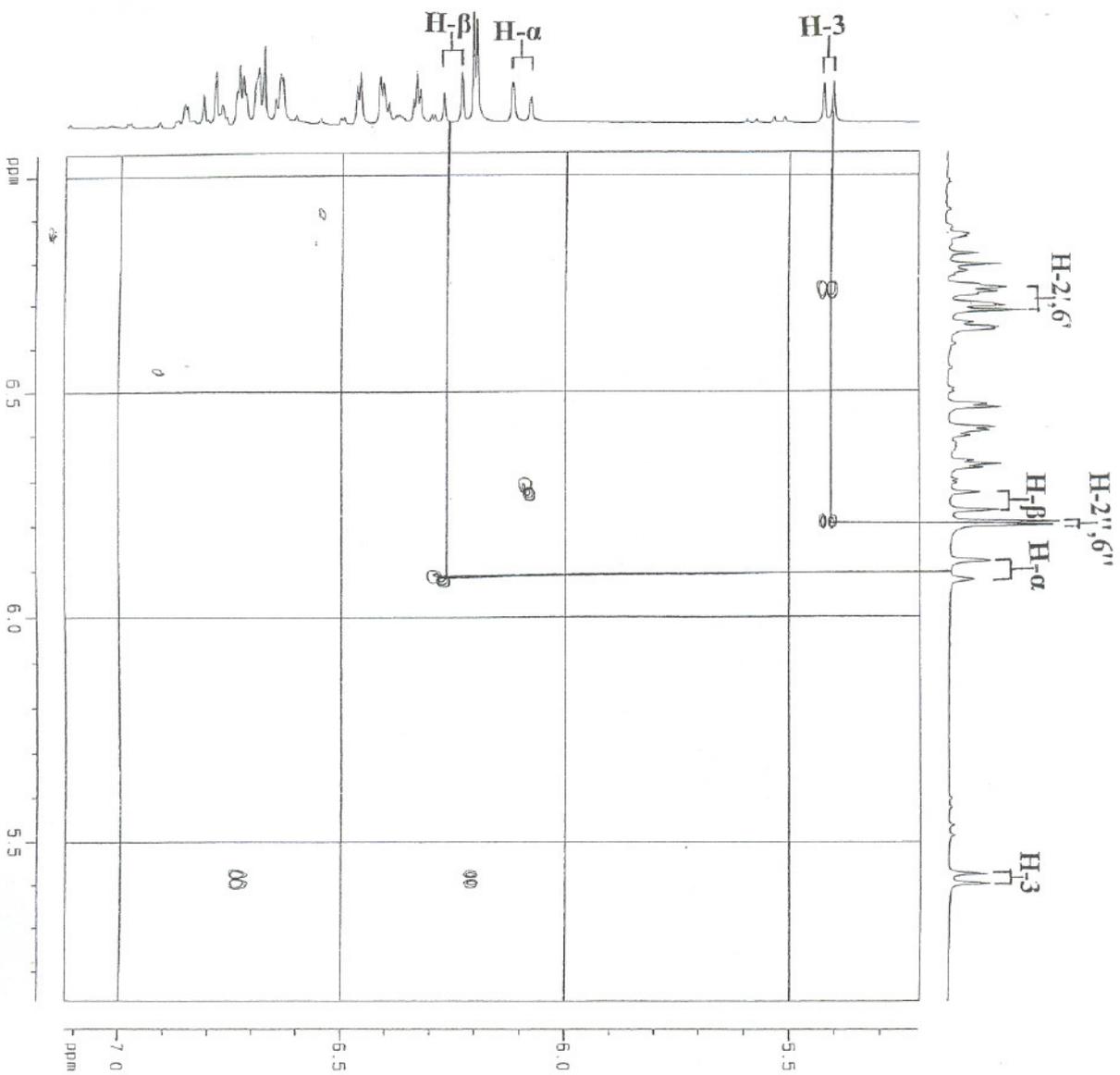
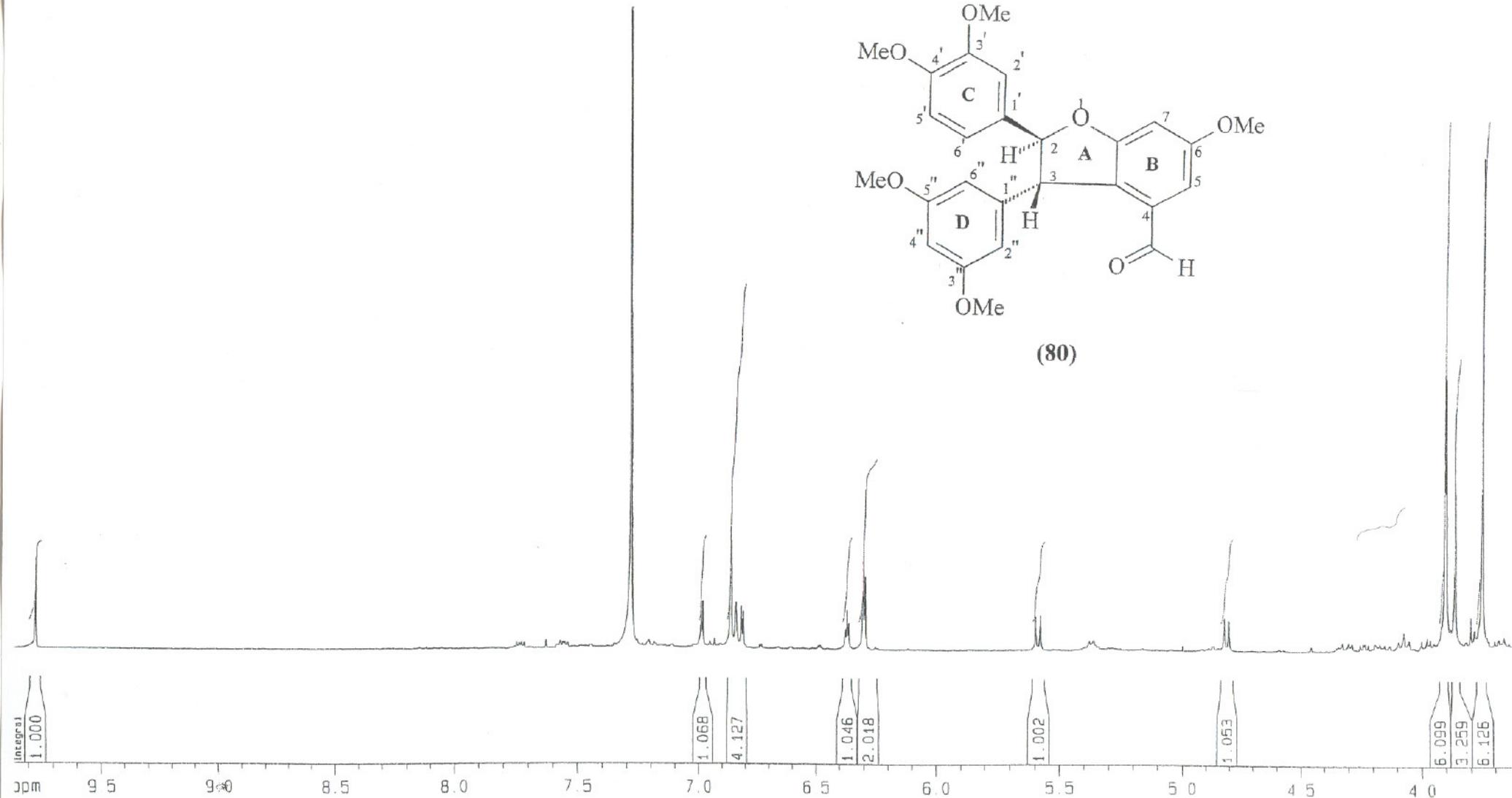
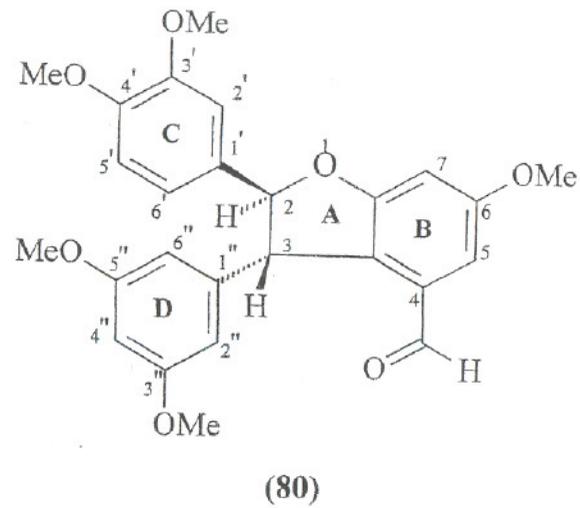


Plate 15: ^1H NMR of *rel*-2,3-*trans*-4-Formyl-2-(3,4-dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran (**80**) [CDCl_3]



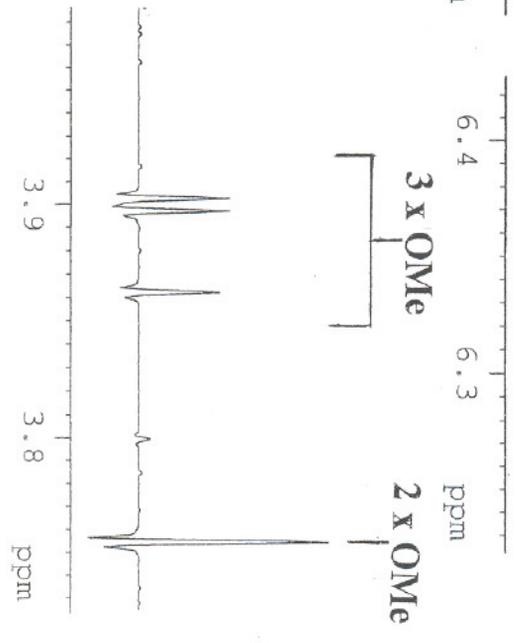
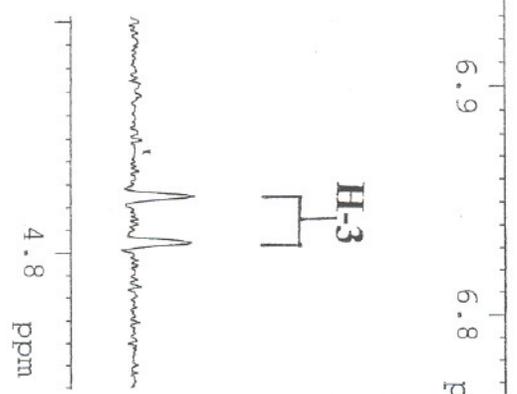
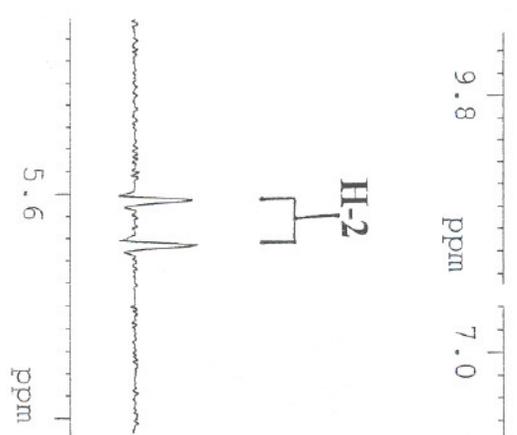
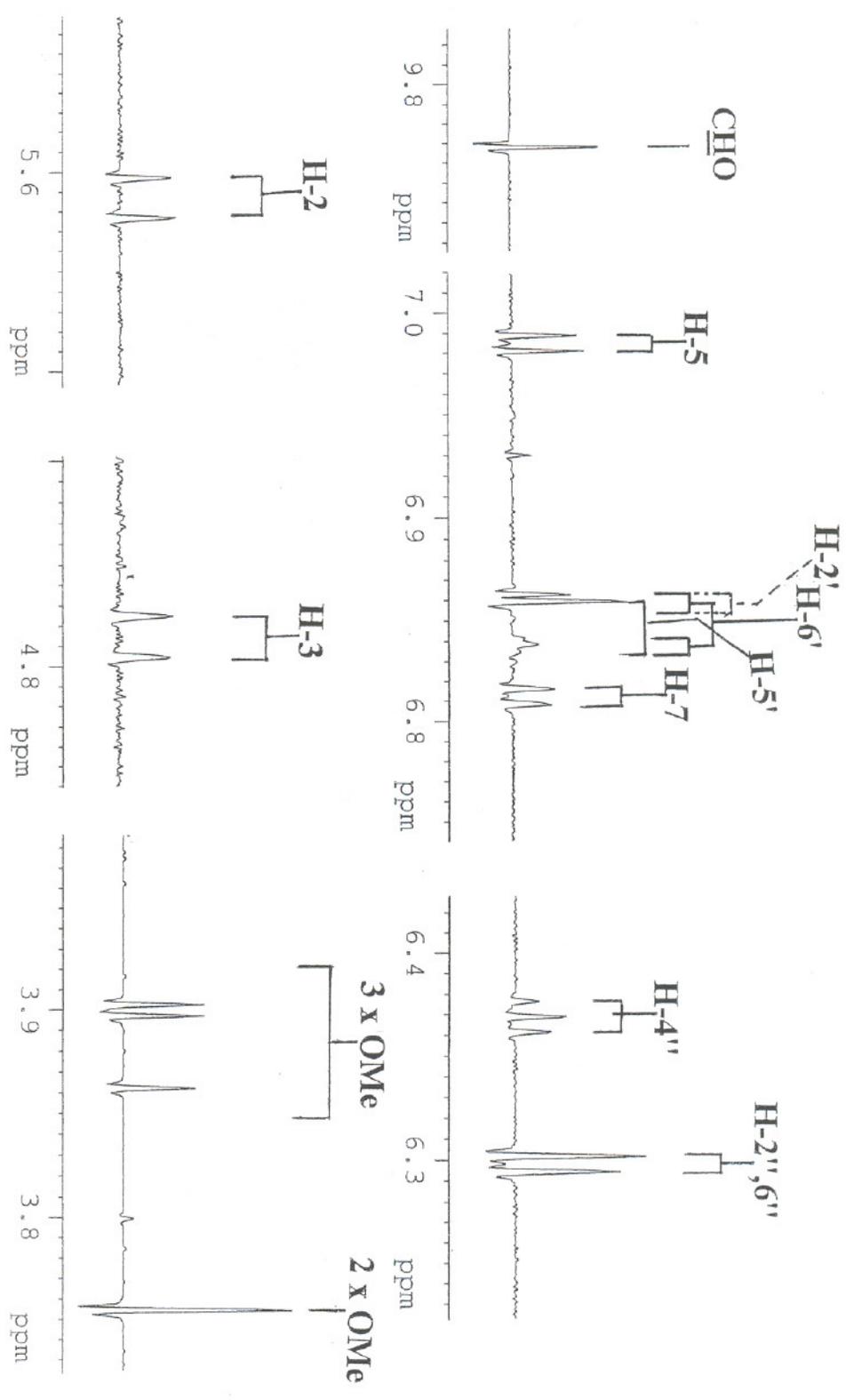


Plate 15a: NOESY of *rel*-2,3-*trans*-4-Formyl-2-(3,4-dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran (**80**) [CDCl₃]

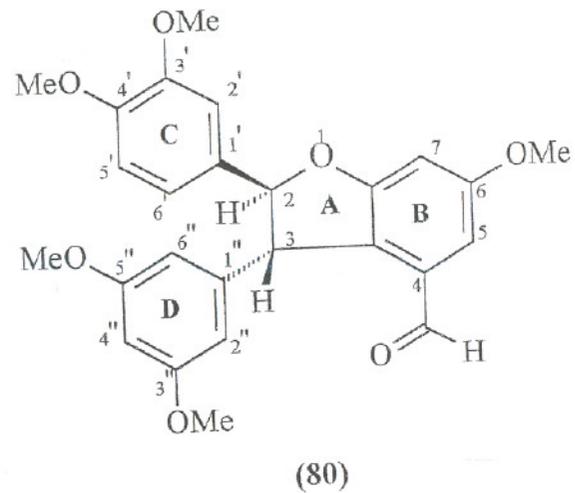
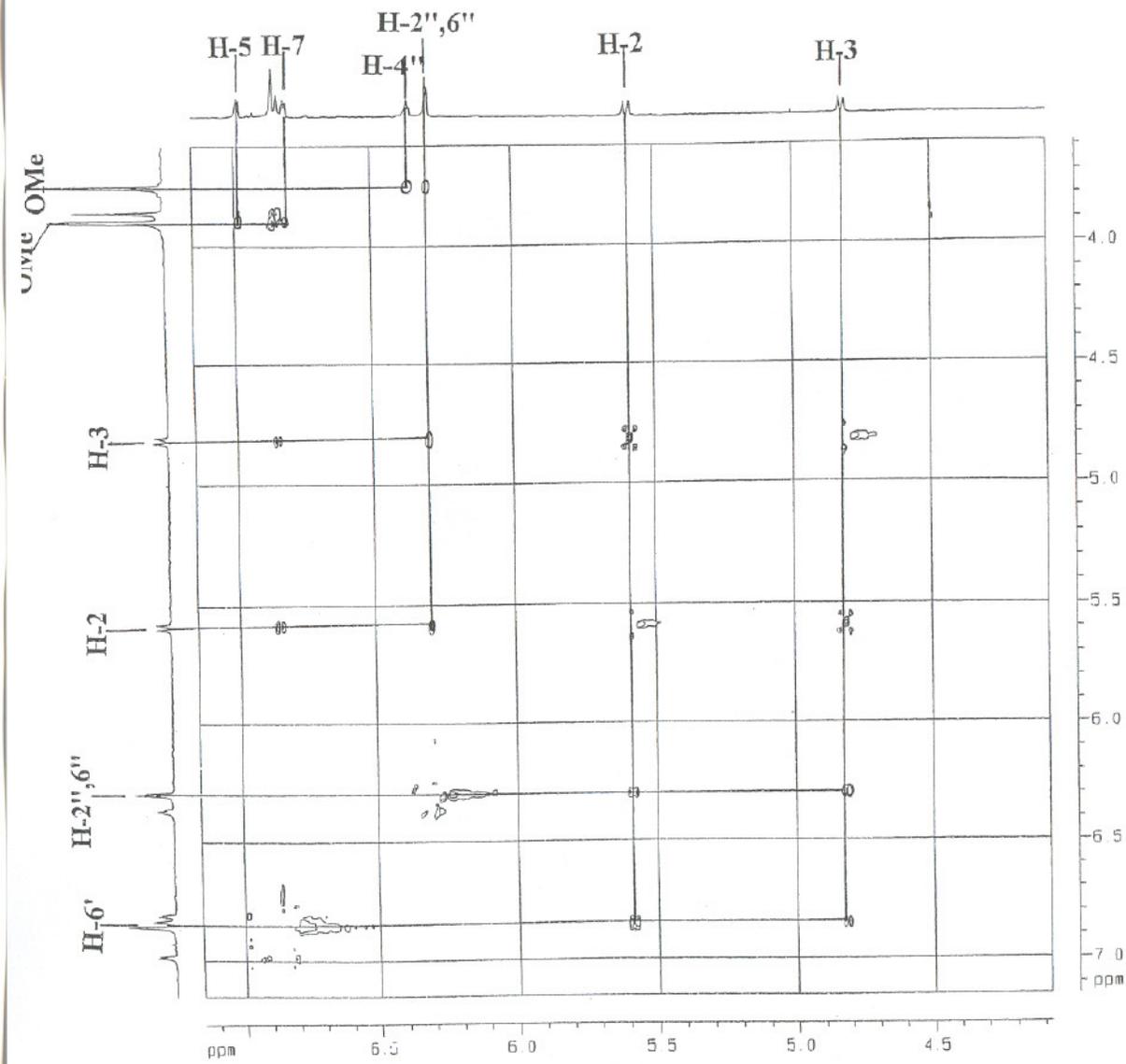
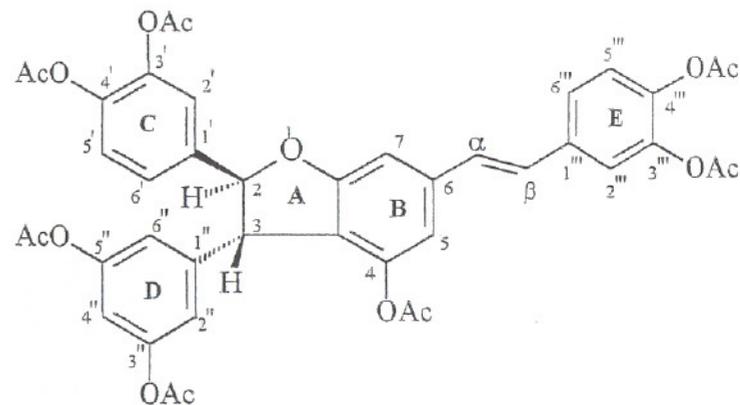
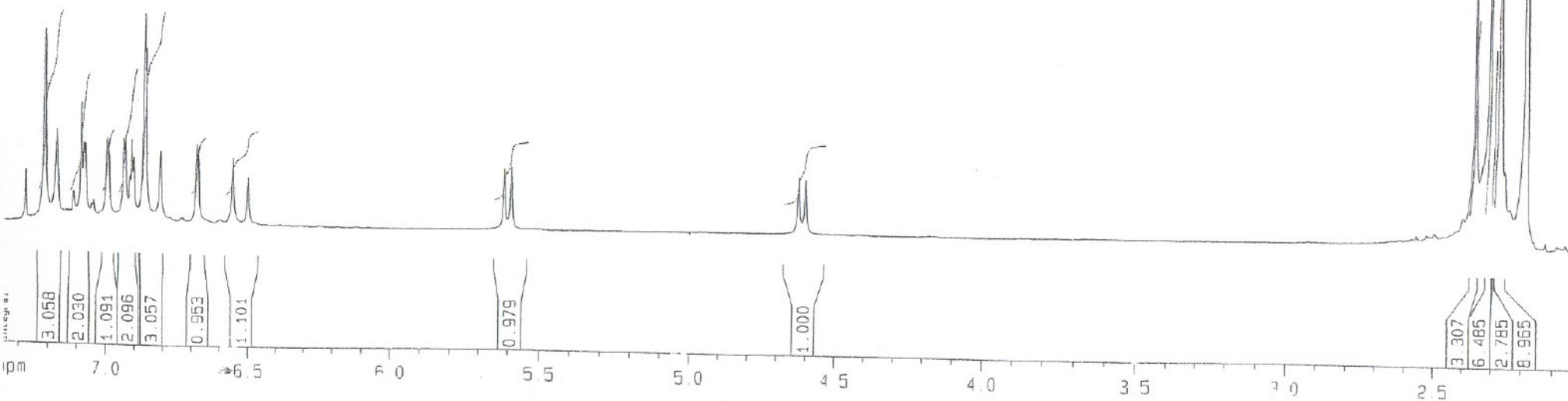
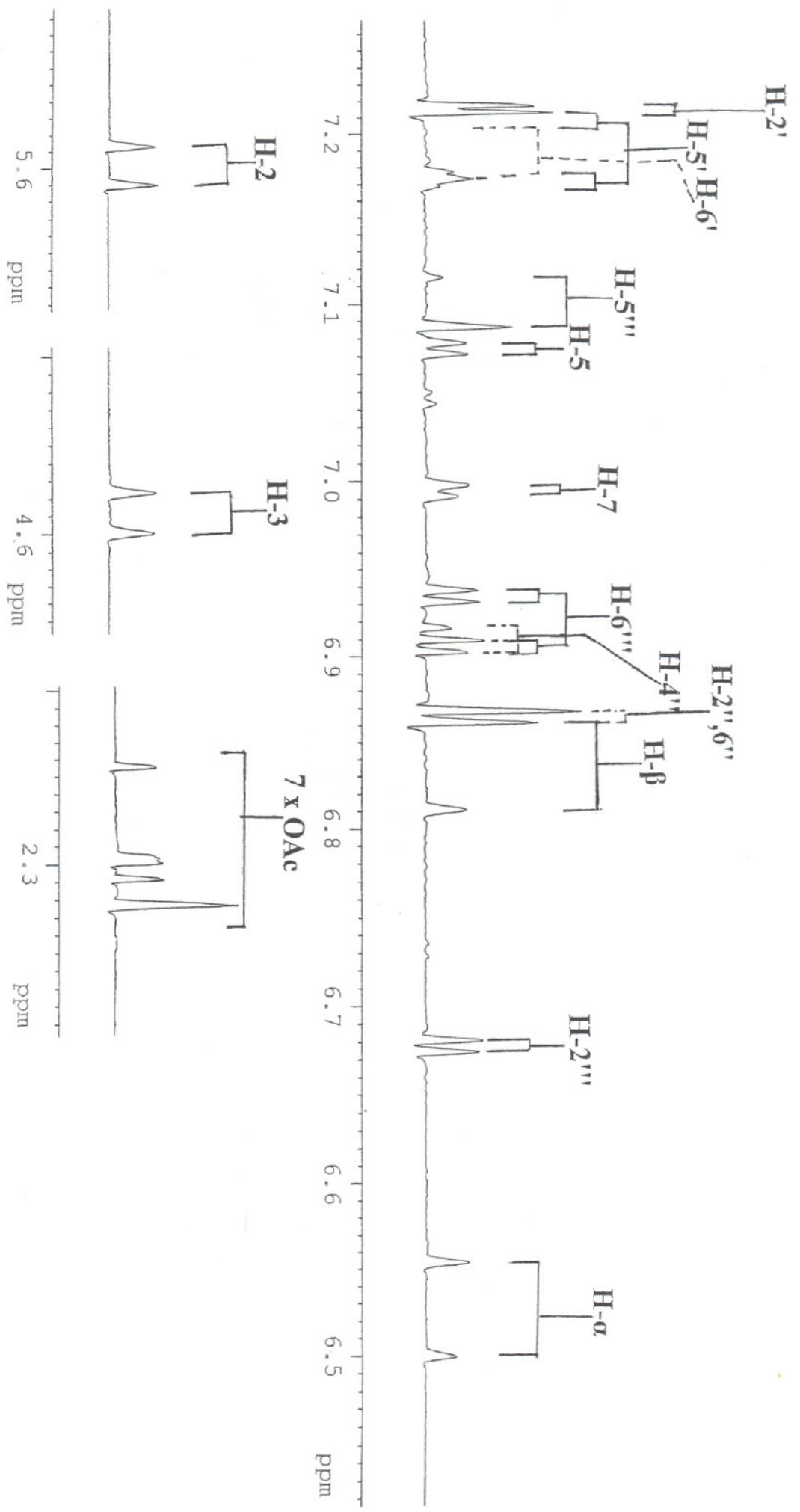


Plate 16: ^1H NMR of *rel*-2,3-*trans*-2-(3,5-Diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-6-[2-(3,5-diacetoxyphenyl)-*E*-1-ethenyl]-4-acetoxy-2,3-dihydrobenzofuran (**81**)
[CDCl₃]



(**81**)





6-[2-(3,5-diacetoxyphenyl)-E-1-ethenyl]-4-acetoxy-2,3-dihydrobenzofuran (81)

[CDCl₃]

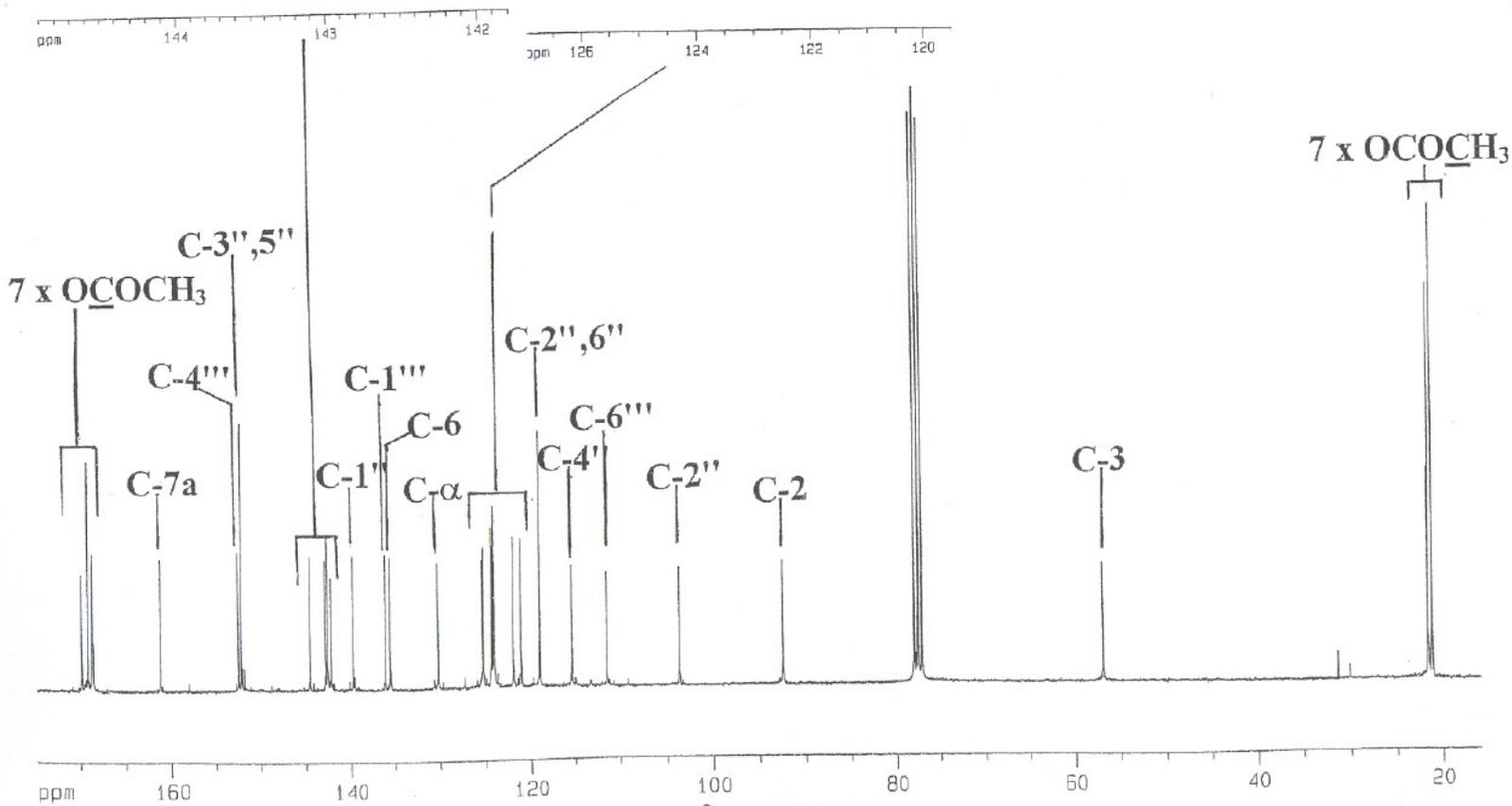
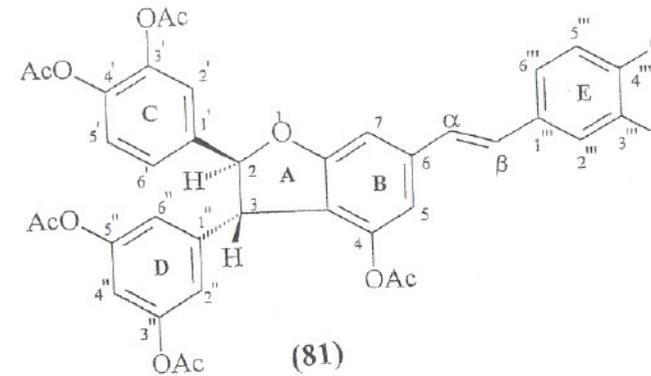
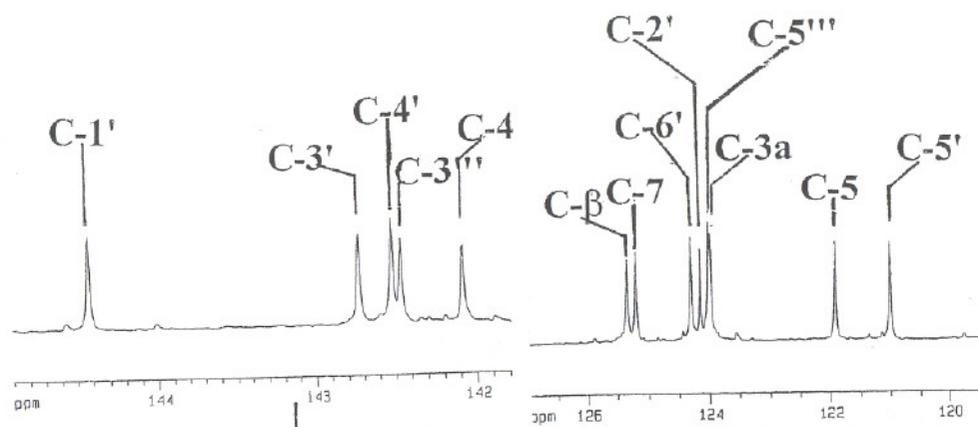
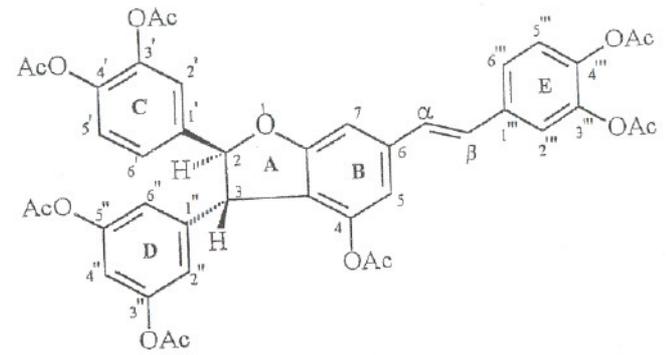
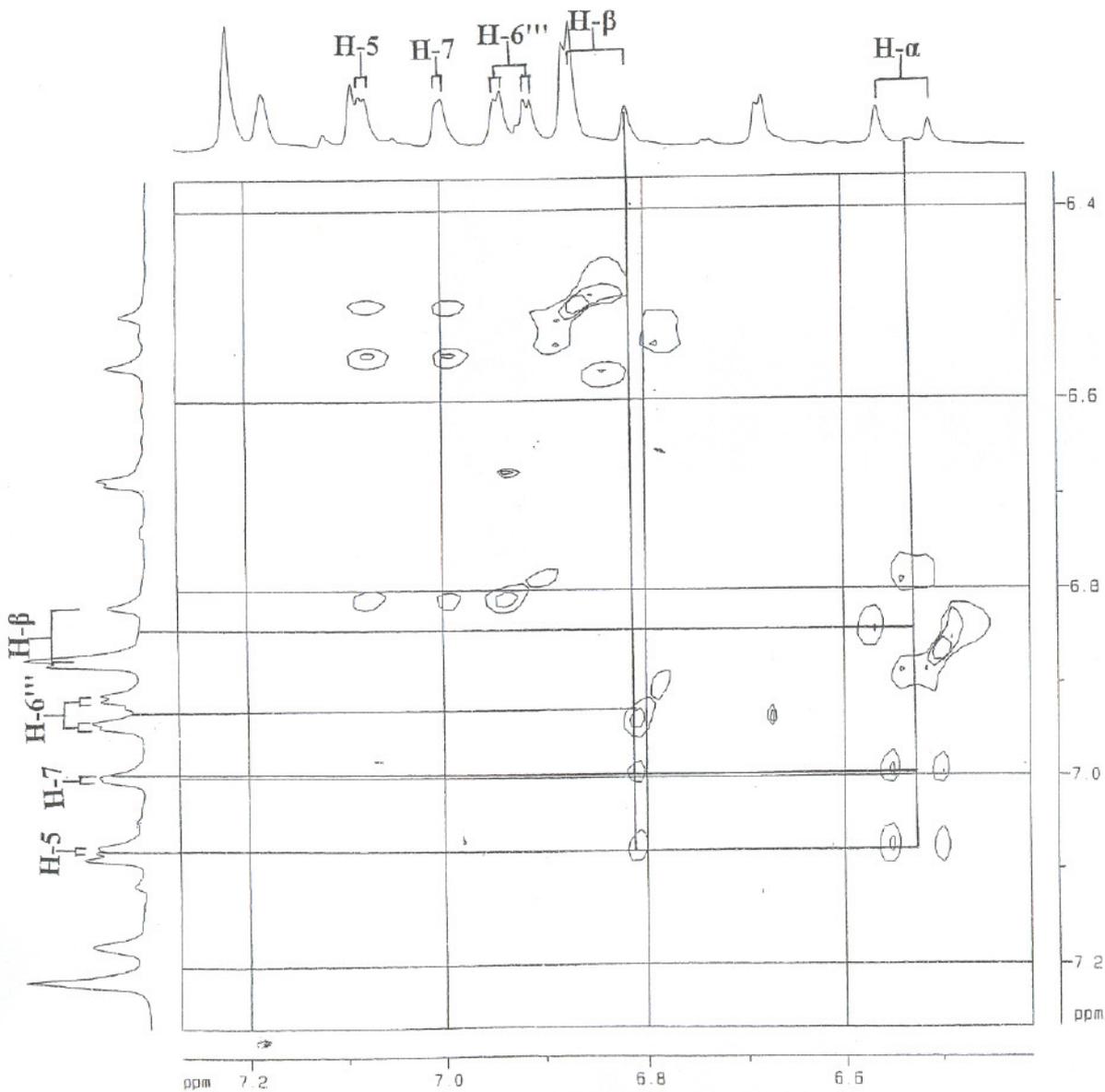


Plate 16b: NOESY of *rel*-2,3-*trans*-2-(3,5-Diacetoxyphenyl)-3-(3,5-diacetoxyphenyl)-6-[2-(3,5-diacetoxyphenyl)-*E*-1-ethenyl]-4-acetoxy-2,3-dihydrobenzofuran (**81**)
 [CDCl₃]



(81)

Plate 17: ^1H NMR of 3,5-dimethoxybenzyl alcohol (**83**) [CDCl_3]

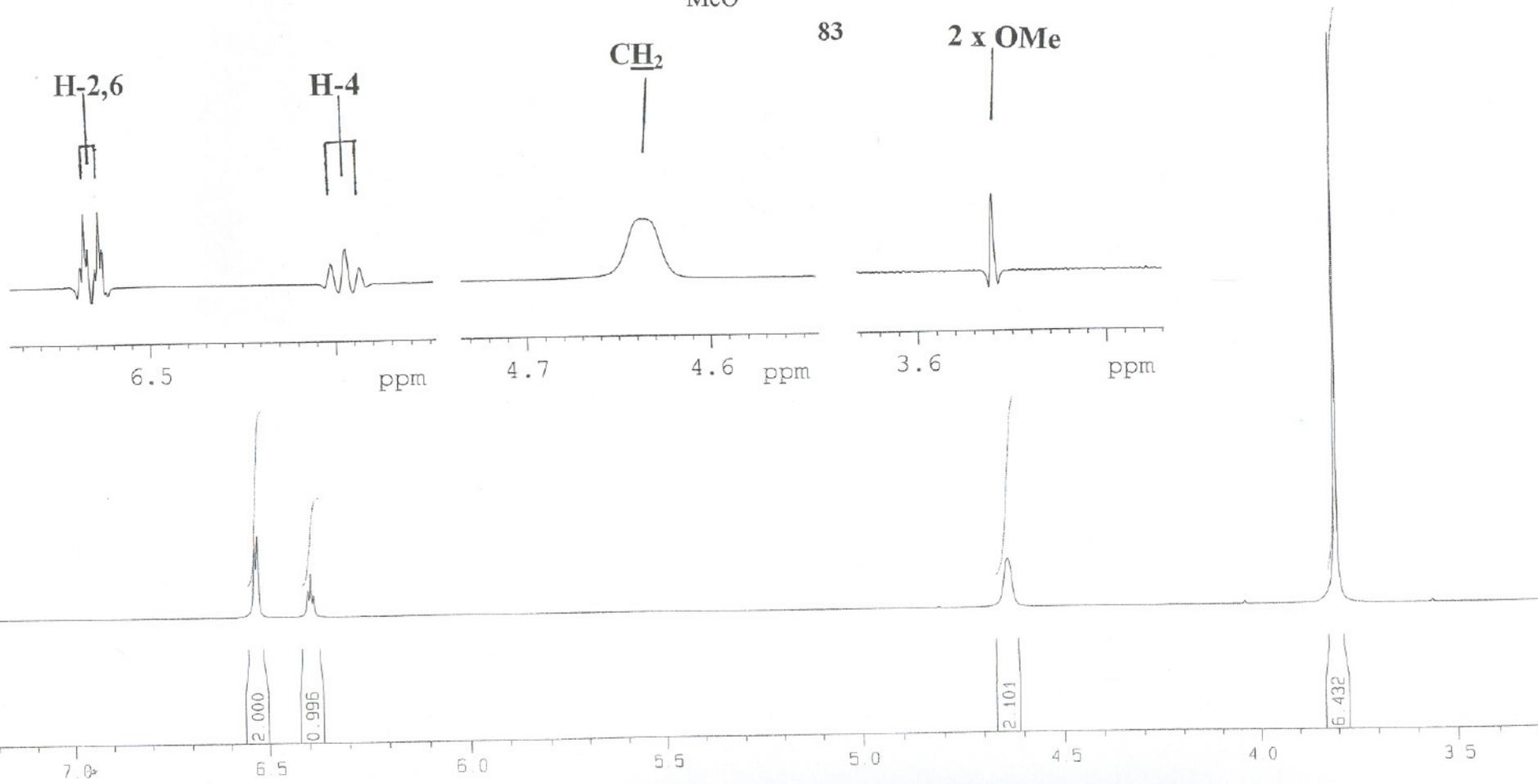
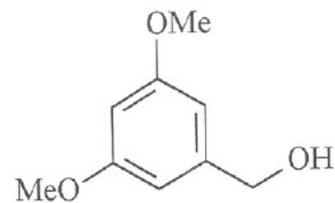


Plate 18: ^1H NMR of 3,5-dimethoxybenzyl bromide (**84**) [CDCl_3]

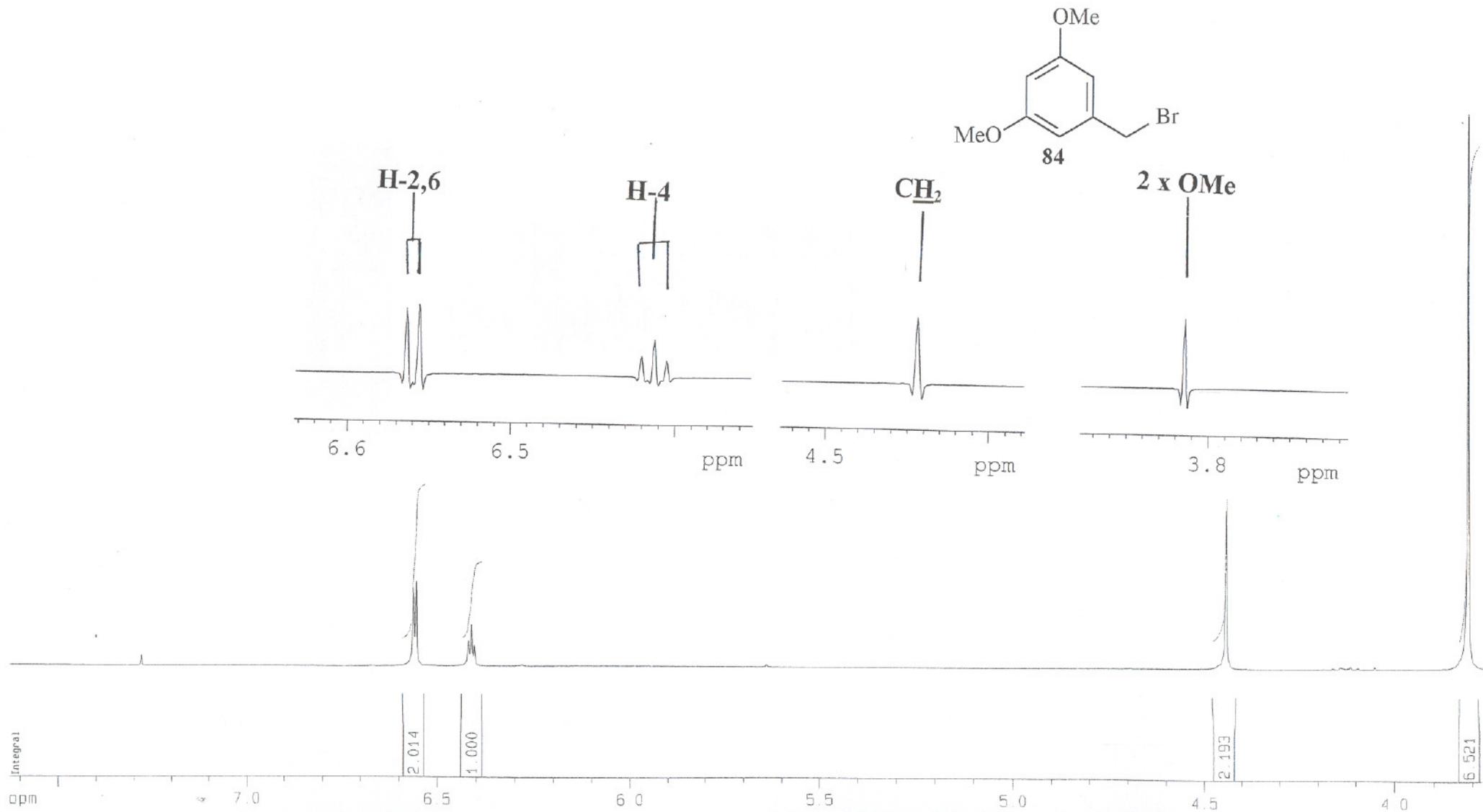


Plate 19: ^1H NMR of 3,5-dimethoxy-triphenyl-benzylphosphonate (**85**) [$(\text{CD}_3)_2\text{CO}$]

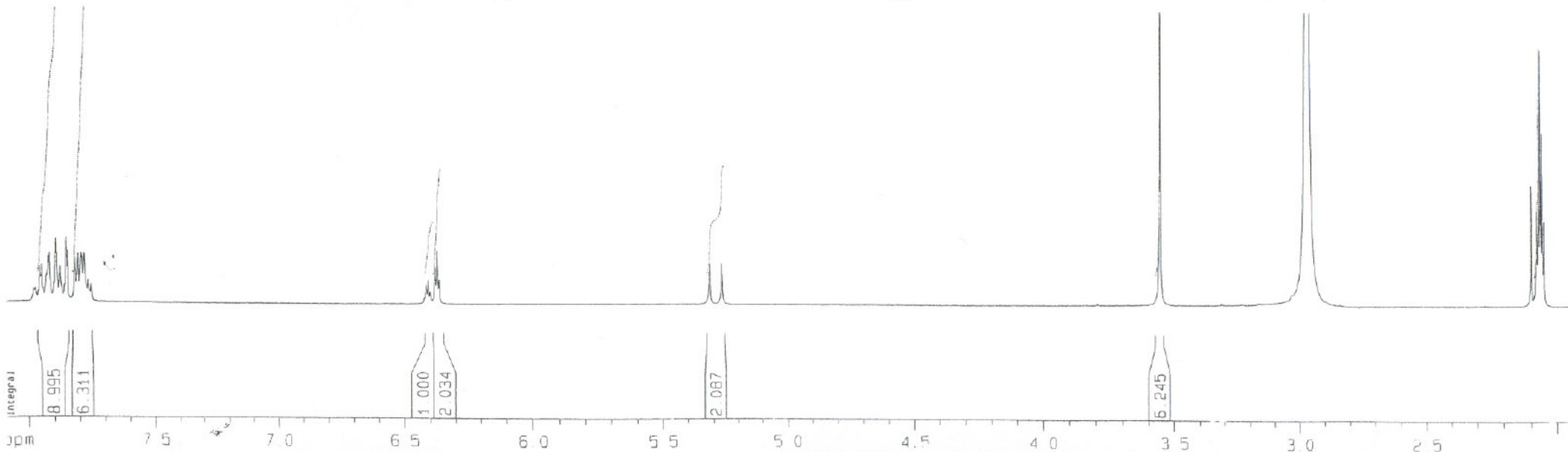
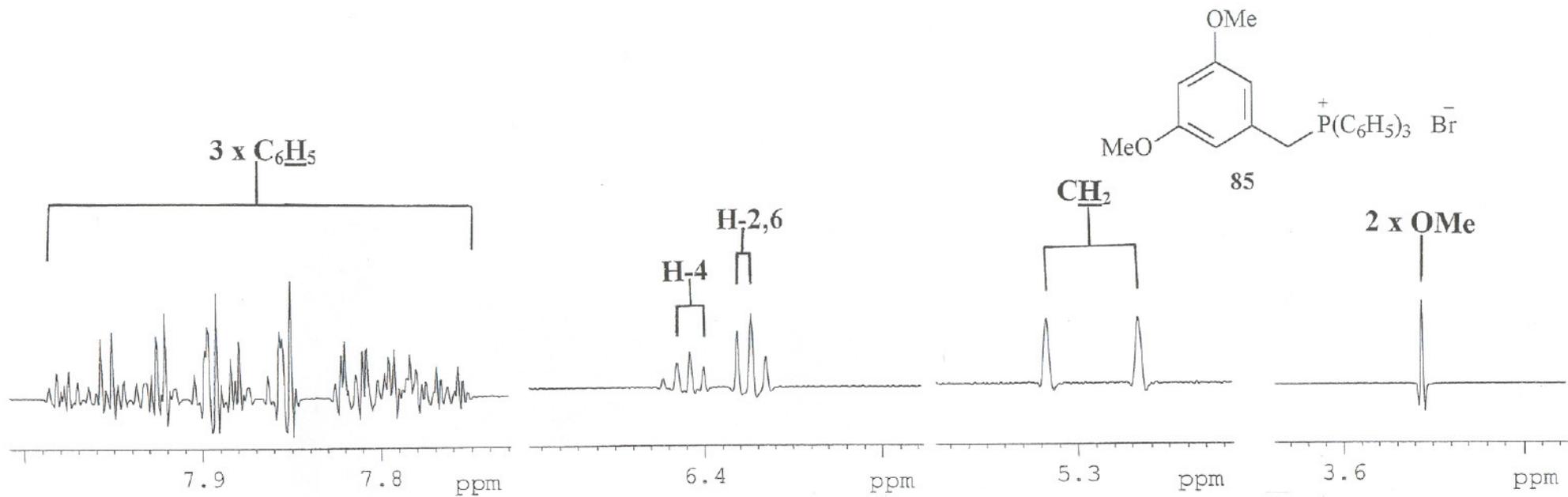


Plate 20: ^1H NMR of 3',4',3,5-tetramethoxystilbene (**87**) [CDCl_3]

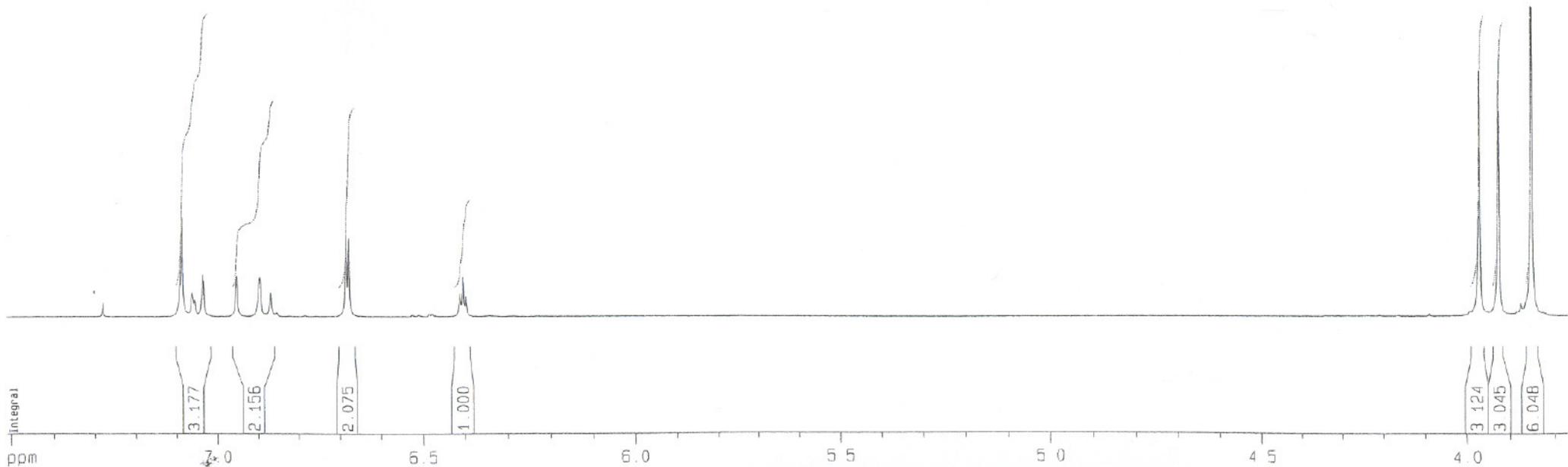
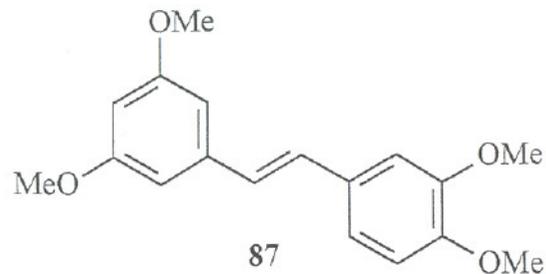
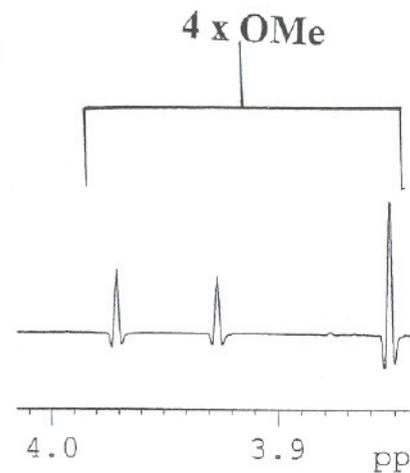
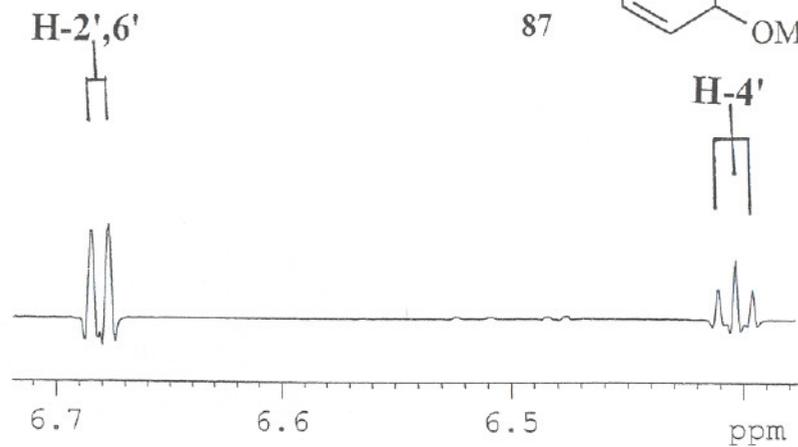
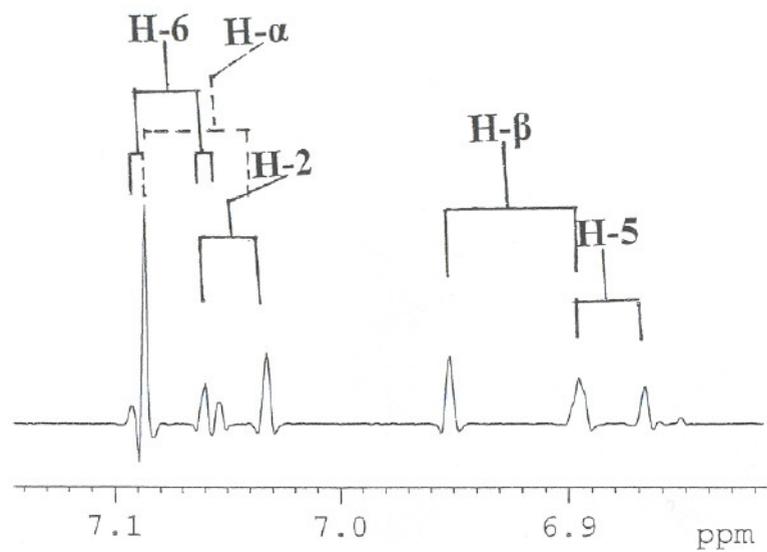


Plate 21. $^1\text{H NMR}$ of Picetannol A (88) [$(\text{CD}_3)_2\text{CO}$]

